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PARASITOLOGY

EDITED BY

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TWENTY-FIVE YEARS AFTER.

A CHRONICLE OF THE DISCOVERIES RELATING TO THE MODE OF TRANSMISSION OF HUMAN MALARIA[1].

BY BATTISTA GRASSI.

THE hypothesis that human malaria is transmitted by mosquitoes was canvassed by Laveran, Manson, Bignami, Dionisi, Koch, and others in 1895–1897—to mention no earlier speculators. But when, and by whom, was this hypothesis converted into a fact?

It is now more than twenty years since Prof. Nuttall attempted to answer these questions by means of a chronological review of the chief researches on this subject—a review to which he has just made a brief but important addition. In 1903 I myself republished, by way of reply, the original papers bearing on the matter, as I hoped that by so doing the question of priority would be settled once for all. But I find that my hopes have been vain, and it is now necessary to state the case anew. I undertake this thankless task once more chiefly because Prof. Nuttall, having assumed the position of judge between Ross and myself, is now appealed to by Ross, Shipley, and many others in justification of their contentions (1)[2].

Here are the exact dates and data:

(A) **18. xii. 1897.**—Ross discovers "pigmented cells" [young zygotes or sporonts, according to present-day nomenclature] in two mosquitoes—possessing spotted wings and eggs which "when not fully developed" are boat-shaped—which had bitten a man with crescents in his blood. Ross had not found the larvae of these mosquitoes (2).

(B) **26. ii. 1898.**—Ross discovers similar bodies in another mosquito with spotted wings [he does not record that he had found the larva of this mosquito] which had been fed on a patient with crescents, and also in a gray mosquito without spots on its wings [as we now know, *Culex fatigans*, closely related to *C. pipiens*] which he had caught while feeding on a patient with benign tertian malaria. He mentions that it may have fed "occasionally on the same man for several days." [It will be recalled that Ross usually made his experiments by liberating the mosquitoes, obtained from larvae, into the mosquito-nets.] This gray mosquito was killed three days later, "when it must have been quite a week old (after hatching from pupa)" (3).

[1] Translated from the Italian by Clifford Dobell.

[2] The notes thus referred to parenthetically in the course of the chronological statement will be found at the end (p. 359 *et seq.*).

(C) **21. v. 1898.**—Discovery by Ross that "*Proteosoma grassii*" of sparrows —shown by Grassi to be related to the parasites of human malaria—undergoes further development in the species of gray mosquito aforesaid. Ross had actually followed the development of the sporonts ("coccidia," as he called them) in the body of this insect. He concludes his report with the statement that "the early stages of similar coccidia have been found [see (A) and (B) *supra*] in mosquitos fed on blood containing two of the parasites of malaria": that is, as is evident from the text of the report, he had found stages in the development of crescents in three mosquitoes with spotted wings, and developmental stages of the benign tertian parasite in one gray mosquito. Consequently Ross was led to incriminate mosquitoes with spotted wings and those with unspotted wings as transmitters of human malaria: and, as a matter of fact, he unreservedly inculpated both kinds of mosquito.

(D) **11. x. 1898.**—Discovery by Ross [after the publication of the note referred to in (E) *infra*] of the transmission of *Proteosoma* by the bite of the gray mosquito. This was based upon his having followed the passage of the sporozoites into the salivary glands, and also upon direct experiments.

(E) **29. ix. 1898.**—Grassi points out that three species of marsh mosquitoes, one of which was *Anopheles claviger* (= *maculipennis*), fall under suspicion as carriers of human malaria. At the same time he exonerates *Culex pipiens*, which is not a marsh form(4).

(F) *Circa* **10. x. 1898.**—Grassi publishes the statement that his servant, who had been bitten in a malarious locality by the three suspected species of mosquito only, has contracted malaria (aestivo-autumnal).

(G) **6. xi. 1898.**—Grassi announces that a healthy individual, who had been bitten by these three species only, and in a non-malarious locality, has contracted aestivo-autumnal malaria. (Experiment made in collaboration with Bignami.)

(H) **4. xii. 1898.**—Grassi states that he has obtained a double tertian infection by bites of *Anopheles claviger* alone in a man who was certainly not previously infected with malaria and who was kept in a non-malarial place. (Experiment made in collaboration with Bignami and Bastianelli.)

At the same time Grassi, Bignami, and Bastianelli announce that they have obtained the first stages of development of the crescents in this *Anopheles*, and that they have also found the first stages of development in two individuals of the same species captured in a room containing malarial patients. [As a result of this second observation (actually first, in order of discovery, as is shown by Charles's letters to Ross), Grassi resolved to determine, if possible, what mosquitoes Ross had used in his experiments: and in attempting to do so he ascertained that *Anopheles* has eggs like those of Ross's mosquitoes with spotted wings.] Consequently, Grassi, Bignami and Bastianelli now express the opinion that the "dappled-winged mosquitoes," in which Ross had found

the pigmented bodies, were probably *Anopheles*: but they do not exclude the possibility that these insects might have infected themselves from animals other than man [since they had not been reared from the larva](5).

(I) **22. xii. 1898.**—Grassi, Bignami, and Bastianelli announce that they have followed the whole cycle of development of the human malaria parasite in *A. claviger*, and that it proves to be very like that described for *Proteosoma* in the "gray mosquito" by Ross.

(K) **5. ii. 1899.**—Grassi, Bignami, and Bastianelli announce that the quartan malaria parasite is also transmitted by *A. claviger*, and that *A. superpictus* can likewise transmit human malaria.

[Grassi having meanwhile ascertained that Ross's "gray mosquito" is almost identical with *Culex pipiens*] Grassi, Bignami and Bastianelli now state that the "gray mosquito" with which Ross believed he had obtained positive results (in tertian malaria) must really have infected itself with the parasite of sparrows. [Grassi had previously said this to Charles, who had informed Ross of it in a letter dated Jan. 6, 1899(6).]

Ross had thus unreservedly incriminated, as a carrier of human malaria, a mosquito with spotted wings ("dappled-winged mosquito") which Grassi—after he had discovered that *Anopheles* transmits human malaria—referred to the genus *Anopheles*; and *Ross had also incriminated a mosquito with unspotted wings* ("gray mosquito") which Grassi, from its close resemblance to *Culex pipiens*, believed to have infected itself really with *Proteosoma* from birds, and not with tertian parasites—as Ross himself had to admit afterwards. It will suffice to add that, as all mosquitoes have wings which are either spotted or unspotted, Ross's data in this respect could not have served as a guide to Grassi or anybody else(7).

(L) **7. v. 1899.**—Grassi, Bignami and Bastianelli announce that *A. bifurcatus* also can carry malaria. They also prove—what they had rather suspected in 22. xii. 1898—that the so-called "brown" or "black spores" of Ross represent degenerate and dead malaria parasites; and they maintain that the parasites are not transmitted to their offspring by infected *Anopheles*, and hence that the whole cycle of development is confined between man and *Anopheles* [like that, for example, of *Taenia saginata* between man and bovines].

(M) **22. vi. 1899.**—Grassi shows that *A. pseudopictus* also transmits malaria. "*It is thus proved that all the Italian species of the genus* Anopheles *propagate malaria* [meaning, of course, *human* malaria]. *And it is quite legitimate to infer from this that all species of* Anopheles, *in every country, are able to carry malaria.*" At the same time Grassi records the results of a long series of experiments showing that mosquitoes not belonging to the genus *Anopheles* do not propagate human malaria—thus confirming what he had already partly proved in collaboration with Bignami and Bastianelli.

(N) **17. ix. 1899.**—Grassi demonstrates the presence of zygotes (oökinetes or vermicules) in the lumen of the stomach of *Anopheles*, and shows that the bite of a single *Anopheles* is sufficient to infect a man.

The problem of the transmission of human malaria was thus definitively solved. What was Ross's share in solving it?

(O) Under date **31. xii. 1898** Ross himself answers this question in a summary of his work (his own words) which was written before he left India and which appeared in the *Annales de l'Institut Pasteur*, February 1899. To appreciate this summary properly it must be remembered that he was kept informed every week by Charles of all the discoveries which were being made by Grassi, Bignami, and Bastianelli: and that Ross, before leaving India, had experimented with two species of *Anopheles* on "two fair cases of tertian fever, and three cases with crescent plasmodia—two of them with crescents in considerable numbers"; but, notwithstanding, always with negative results. In his summary, however, he limits himself to the statement that in 1897 he obtained the pigmented bodies in two mosquitoes of a new species—without mentioning that they were *Anopheles*. He then reviews his own experiments with *Proteosoma*, remarking that the intermediate hosts of the human malaria parasites ought to be precisely determined, and that the investigation promised some interesting discoveries: and he adds that the "black spores" are evidently resistant stages, capable of survival outside the living organism, and that he therefore thinks it very probable that the malaria parasite can reproduce itself in the outside world, without the necessity of passing into the blood of man. He records that Grassi, "working quite independently," has recently made patient epidemiological investigations which lead him to suppose that one species of mosquito, *A. claviger*, is the transmitting agent of malaria in Italy; and that Grassi, Bignami, and Bastianelli have succeeded in producing the "pigmented bodies" in *Anopheles* fed on men infected with crescents. Finally he concludes "I consider it probable that malaria is communicated to man solely by the bites of mosquitoes, and perhaps of other insects" (8).

(P) Up to 9. ix. 1899 Ross published no further researches. But from **9. ix. 1899** to **14. x. 1899** he announced that he had found human malaria parasites in two African species of the genus *Anopheles*.

The reader may now judge for himself whether Ross's work in India may justly be said to have proved that *Anopheles* is the intermediary in the conveyance of malaria to man—as is said, for example, by E. J. Wood (*American Journ. Trop. Med.* II. 363, 1922).

If it be asked why Ross did not succeed in the enterprise to which Manson (3. x. 1898) incited him—that is, "to grab the discovery [of the transmission of human malaria] for England"—the answer is to be found in Ross's method of experimentation. He made trials at random with various blood-sucking insects, and after letting them bite malarial subjects attempted to find developmental stages of the parasite in them. Consequently he had to trust

largely to chance: and since there are at least three malarial parasites of man and the blood of an infected human being is not invariably infective for mosquitoes—which do not become infected, moreover, if the temperature is too low; and since also there are numerous kinds of mosquitoes, Ross's task was not unlike that of Sisyphus. Nevertheless, he succeeded in the case of the sparrows, because these birds—when infected—are fortunately almost always in a condition to infect the "gray mosquito"—the commonest species in the place where Ross performed his experiments.

Grassi, on the other hand, had some years before indicated—in connexion with his work on the intermediate hosts of helminths—the necessity of provisionally incriminating the probable transmitting agents (method of limitation to suspected hosts), with which experiments could then be profitably begun: in much the same way as the police, in trying to find the author of a crime, begin by laying their hands upon the suspects—not by examining the general population. Also, in putting the suspected mosquitoes to the proof Grassi proceeded in a different manner from Ross. He first ascertained whether the bite of the insect produces malaria, and he searched for the parasite in the body of the mosquito afterwards. And naturally he did this because the minute parasite might have evaded detection by the microscope, whilst the discovery of its mode of transmission could, from a practical standpoint, have been equally well made even if its method of development in *Anopheles* had remained entirely unknown.

For further particulars and references see:

GRASSI, B. (1903). *Documenti riguardanti la storia della scoperta del modo di trasmissione della malaria umana.* (Milano.) [I still have some copies of this, which will be sent to those who desire them.]

—— (1924). The transmission of human malaria. *Nature*, March 1, p. 304.

ECKSTEIN, F. (1924). Zur Entdeckungsgeschichte der Malaria. Eine historisch-entomologische Studie. *Zool. Anz.* Bd. 58.

NOTES.

(1) See Nuttall, G. H. F. (1901). On the question of priority with regard to certain discoveries upon the aetiology of malarial diseases. *Quart. Journ. Micr. Sci.* XLIV. 429. See also the same writer's recent note in *Parasitology*, XVI. No. 2, 220–221. 1924.

(2) Prof. Nuttall says, on the contrary, that "the insects were bred from larvae." This cannot be so, for in his paper Ross himself says that he was unable to find the larvae. Moreover, Ross writes in his *Memoirs* (p. 223) that the mosquitoes with which he experimented had eggs which were "large and black, boat-shaped with central spines"; and this proves decisively that they could not have been "recently hatched out in the original bottle," but must have fed on blood two or three days previously and must therefore have been taken in the winged condition.

(3) Prof. Nuttall says that this insect (*Culex*) was not raised from the larva. This is not stated in Ross's paper. He says it "was observed feeding on a patient....I judged for many reasons that it had been feeding occasionally on the same man for several days. It was killed three days afterwards, when it must have been quite a week old (after hatching from pupa)." From this I had concluded that, like all the other "gray mosquitoes" used by Ross for his experiments, this insect had been bred from the larva and then liberated into the mosquito-net protecting the patient; so I therefore thought it necessary to ask Ross afterwards how he had determined the age of this mosquito, if he had not raised it from the larva. Ross replied that he had judged the

age from the size of the eggs: to which I objected that this was impossible, since mosquitoes do not lay their eggs once only—as Ross himself had shown. (In the *Report of the Malaria Expedition* (1900) it is noted that "naturally-fed gnats *invariably* laid eggs after two or three days..." and that "previously fed and fertilized insects would lay a second batch of eggs after a second meal of blood." "These observations are wholly in accord with the results of the prolonged study of many kinds of gnats made in India by one of us.") In his *Memoirs* Ross reverts to the subject; but forgetting that he had written "when it must have been quite a week old" he now tries to show instead that "in certain circumstances I [Ross] can guess the minimum age of a mosquito!"

In short, it is indeed a fact that Ross has many times asserted—but *too late*, and *only after I raised my objections*—that the "dappled-winged mosquitoes" (determined as *Anopheles* by me) *were* reared from the larvae, and that the "gray mosquito" (determined by me as a *Culex* very like *pipiens*) *was not* reared from the larva.

The equivocation appears again in the two following quotations. In the *Report* of 1900 it is said (p. 30): "It is noteworthy that all the successful experiments made by one of us in India, without exception, were made with insects fed in mosquito-nets in the presence of males; while his negative experiments with various species of *Anopheles* fed on human subjects were nearly all made with females isolated in test-tubes, and therefore not fertilized." Compare this with the following passage from the *Memoirs* (pp. 221–222): "The worthy Hospital Assistant ran in to say that there were a number of mosquitoes of the same class [*i.e. Anopheles*] which had hatched out in the bottle that my men had brought me yesterday. Sure enough there they were: about a dozen....Immediately my patient, Husein Khan, a man with fairly numerous crescents (and also *Filariae*) was stripped and put on the bed under the mosquito-net. This was at 12.25 p.m. by my notebook; and in five minutes ten of the new mosquitoes had gorged themselves on him and were caught by the Hospital Assistant, each in its separate test-tube." These were the insects which gave Ross the positive results recorded in (A) above, under date 18. xii. 1897. But, the reader will ask, was not the presence of males necessary in order to obtain positive results? Ross has forgotten about that!

(4) On this note Prof. Nuttall has written the following comments in his chronological account: "In his paper in the 'Policlinico' (October 1st, 1898), Grassi writes*: 'In conclusione, io sono d' avviso che il *Culex penicillaris* e l' *Anopheles claviger* o per lo meno il *Culex penicillaris*, fors'anche il *Culex malariae*, nella malaria si comportano come la zecca nella febbre del Texas.' Grassi therefore makes a misstatement in a later paper (December 1st, 1900) when he writes†: 'Proclamai come indiziati due specie di *Culex*, ma sopratutto l' *Anopheles claviger*.' It is curious that Grassi should subsequently have continued to lay stress upon the geographical coincidence having led him to the discovery of *Anopheles claviger* being a host of malarial parasites, for two out of three species which he for this reason supposed must be hosts were afterwards proved not to be such. He certainly considered *A. claviger* at first to be of quite secondary importance; we have his own words for it‡: 'Certi casi di malaria sviluppatisi in settembre a Locate Triulzi, nei quali gli *Anopheles* di certo o non punsero o soltanto rarissime volte, *denunciano decisamente come trasmissore il Culex penicillaris, enormemente comune in tutti i luoghi malarici*.' (The italics are Grassi's.) It is but fair to Ross to state here that Grassi in his paper of the 1st of October refers to the experiments made by Smith and Kilborne upon Texas fever, and by Ross upon avian malaria as having been a 'forte argomento' in favour of the mosquito-malaria hypothesis. In the paper read on the next day at the Accademia dei Lincei, under the same title as that which appeared in the 'Policlinico,' Grassi omits to mention Ross, though he refers to what was known regarding Texas fever. The paper, published in the 'Transactions' of the Accademia, differs in several respects from that which appeared in the 'Policlinico.'"

* [Translation.] In conclusion, I am of the opinion that *Culex penicillaris* and *Anopheles claviger*, or at least *Culex penicillaris* and perhaps also *Culex malariae*, behave in malaria as the tick does in Texas fever.

† [Translation.] I announced that two species of *Culex* were indicated [as suspect], but more especially *Anopheles claviger*.

‡ [Translation.] Certain cases of malaria which occurred in September at Locate Triulzi—where *Anopheles* was certainly not biting, or bit only very seldom—*definitely denounce Culex penicillaris, which is excessively common in all malarious places, as a transmitter* [of human malaria].

To this I reply:

(i) That my note in the *Policlinico* is a second edition, with additions, of that in the *Lincei*—and not *vice versa*, as Prof. Nuttall supposed. The confusion arose (*a*) from Prof. Nuttall having inadvertently (for he knows Italian quite well) mistranslated the words "Comunicazioni pervenute all' Accademia prima del 2 ottobre" [*i.e.* communications which reached the Academy *before* October 2] as "paper read on the next day [Oct. 2] at the Accademia": (*b*) from Prof. Nuttall not having known that the *Policlinico* (Sezione medica) in which the second edition of my note in the *Lincei* appeared, although it bore the date October 1, really came out several days later, and that the editor refused to allow me to add the words "second edition" to my note; (*c*) from Prof. Nuttall not having justly appreciated the textual differences between the note in the *Lincei* and that in the *Policlinico*. For example, he must have inferred—if his view were correct—that in my note in the *Lincei* (not the second, but really the first edition) I had suppressed the addendum recording the infection of my servant (see (F) in the present chronicle) which was published in the version in the *Policlinico* (really the second, not the first edition): which would have been absurd.

It is true that I did not mention the researches of Ross in what was really the *first* edition; whereas I did mention them in the *second* edition—that is, when I became aware of them. Yet this is the very opposite of what Prof. Nuttall avers, and surely, therefore, "it is but fair to" Grassi to say so.

(ii) That nobody who reads my note without prejudice can infer from it what Prof. Nuttall did in 1901. He takes note of what I said about *Culex penicillaris*, but entirely forgets—I say *forgets*, because in the *Centralbl. f. Bakt.* 1899, he recollected it perfectly—that nearly three pages of my paper are devoted to *Anopheles claviger*, of which I wrote*: "*Si trova invece costantemente in tutti i luoghi malarici e più frequentemente nei luoghi, in cui la malaria è maggiore, una grossa zanzara che molti denominano anche zanzarone, o moschino.* Esso vien appellato dai zoologi *Anopheles claviger* Fabr....Questa specie...può definirsi *vero indice, vera spia della malaria.* I rapporti fra l' *Anopheles claviger* e la malaria sono così sorprendenti che veramente impongono e conducono alla persuasione che tra di loro debba esistere un nesso. Si può aggiungere che l' ambiente palustre è necessario allo sviluppo di questa specie....Il rapporto intimo fra l' *Anopheles claviger* e la malaria è stato da me verificato per molte località della Lombardia, del Veneto, delle Maremme Toscane e anche per alcune località della Campagna Romana, ecc....Riassumendo: zanzarone (*Anopheles claviger*, sinonimo di *A. maculipennis* Meigen) e malaria sono intimamente connessi. Nell' epoca in cui trovai enormemente diffuso lo zanzarone, predominava moltissimo la terzana ed io perciò lo metterei in rapporto colla terzana."

After saying this—and much else, which I omit for the sake of brevity—I was surely entitled to write, as I did in December 1900, that I incriminated two species of *Culex*, but more especially *Anopheles claviger*. It is very true that I also greatly suspected *Culex penicillaris*, and wrote that "il *C. penicillaris* vuolsi considerare spia della malaria, come l' *Anopheles claviger*, specialmente se le osservazione si fanno, invece che in luglio e in agosto, in settembre"; but it is undoubtedly incorrect to say with Prof. Nuttall that I "certainly considered *A. claviger* at first to be of quite secondary importance." Moreover, Prof. Nuttall does not take into account the fact that Locate Triulzi is in Lombardy, where the malarial season is almost over by September—whence the people there call malaria "August fever." However, my views were fully set out in another paper

* [Translation.] On the other hand, in all malarious places—and more frequently in places where malaria is more prevalent—there is constantly found a big mosquito (*zanzara*) which many people also call a *zanzarone* or *moschino*. This has been named *Anopheles claviger* Fabr. by zoologists....This species...may be characterized as the true sign—a true index—of malaria. The relations between *Anopheles claviger* and malaria are so surprising that they actually tempt and even induce one to believe that the two must be connected. It may be added that a marshy environment is necessary for the development of this species....The close relation between *Anopheles claviger* and malaria I have verified for many districts in Lombardy, Veneto, the Tuscan Marshes, and also for some parts of the Roman Campagna, etc....To sum up: Mosquitoes (*Anopheles claviger*, a synonym of *A. maculipennis* Meigen) and malaria are intimately connected. At the time when I found this mosquito excessively prevalent, tertian fever very greatly predominated: and therefore I would connect it with tertian.

published in the supplement to the *Policlinico* in 1900—which seems to have escaped Prof. Nuttall. This paper was entitled "Cenni storici sulle recenti scoperte intorno alla trasmissione della malaria," and in it I said (these are my exact words)*: "Partendo dall' osservazione che vi sono luoghi non malarici con molte zanzare, dopo confronti fatti nelle più svariate parti d' Italia, conchiusi che dovevano ritenersi sospetti l' *Anopheles claviger* e due *Culex*. Nella Nota in cui si riferiscono questi fatti (*fine di settembre* 1898) per la prima volta viene dichiarato che l' *Anopheles claviger può definirsi vero indice, vera spia della malaria* e che *questa e quello sono intimamente connessi*. Evidentemente la detta Nota ha aperto una nuova strada maestra che in meno di due mesi condusse a scoprire quanto Ross e altri aveva tentato invano da anni."

Prof. Nuttall, who was in close touch with me for some months, will remember that I did think, at one moment, that I was on a false scent. But this happened later—that is to say, after I had made my preliminary inquiries—and was a result of my hearing from Koch, who was in Rome all that time, that *Anopheles* certainly does not carry malaria. This judgement of Koch's influenced, to some extent, what I said about those cases in which all three of the suspected species of mosquito came into consideration (the case of my servant, and the experiment made with Prof. Bignami); but my doubts did not last very long—which shows, also, that I was not "entirely guided by Ross." However, the really important point was—as anyone can see—my ascertaining that *at least one of the three species incriminated must be guilty*. To demonstrate which of them it was, or whether they all were, was a work that anybody could carry out afterwards: it was only necessary to get the different species to bite patients separately.

In the chronicle under discussion, shortly after the passage quoted above, Prof. Nuttall, not taking into account the fundamentally important circumstance that I had initiated an inquiry which would give a rational and not a random direction to my experiments, uses as an argument against me the fact that "The coincidence in the geographical distribution of ague and malaria-bearing mosquitoes in Italy, as claimed repeatedly by Grassi, has been disproved by Celli." But granting the fact (which was noted by Celli, but which I had already indicated for a much more restricted area) that there are places where malaria has disappeared though the *Anopheles* remain, what real bearing has it upon the question at issue? To refute my observations it was necessary to show that they were not correct for the places indicated—a thing which Celli never even thought of doing. I could cite several passages from his book, *La malaria secondo le nuove ricerche* (1900), in support of this. For example, he says†: "Il Grassi dimostrò che le zanzare capaci di ospitare i parassiti malarici e d' infettar l' uomo appartengono al genere *Anopheles*." "Il Bignami cercava già da tempo di far pungere uomini sani colle zanzare raccolte in siti malarici, zanzare che si vide poi essere del genere *Culex*. Ma non riusciva a riprodurre la febbre. Venne poi il Grassi a suggerire di far pungere dagli anofeli, e così per la prima volta si riprodusse nell' uomo la febbre malarica a

* [Translation.] Starting from the observation that mosquitoes are plentiful in non-malarious places, and after comparing the most diverse districts of Italy, I came to the conclusion that *Anopheles claviger* and two species of *Culex* must be regarded as suspect. In the note in which these facts are recorded (end of September, 1898) it was stated for the first time that *Anopheles claviger* can be characterized as a true index, a true sign, of malaria: and that the one is intimately connected with the other. It is obvious that this note opened up a new main road which led, in less than two months, to discoveries such as Ross and others had been vainly seeking for years.

† [Translation.] Grassi showed that the mosquitoes which are able to act as hosts for the malaria parasites, and which can infect man, belong to the genus *Anopheles*.—Bignami had already tried for some time to get mosquitoes caught in malarious places to bite healthy human beings—mosquitoes which were afterwards found to belong to the genus *Culex*: but he did not succeed in reproducing the fever. Grassi then came forward with the suggestion that *Anopheles* should be made to bite instead: and as a result malarial fever (the aestivo-autumnal form) was produced in man for the first time. Afterwards, by the same means, the spring form of tertian was successfully produced.—Where there is malaria, mosquitoes abound: but contrariwise, malaria does not occur in every place where mosquitoes are plentiful. This fact was elucidated by Grassi, who has carried out an accurate medico-zoological investigation of the mosquitoes all over Italy—both in malarious districts and in those which are free from malaria: and he has demonstrated that in the malarious places particular species of mosquitoes occur, which are absent in places that are immune.

forma estivo-autunnale, e poi si riuscì a riprodurre con lo stesso mezzo anche la terzana primaverile." "Dove c' è malaria, abbondano le zanzare: ma viceversa, non in ogni luogo ove abbondono zanzare vi è malaria. Questo fatto venne spiegato dal Grassi, il quale ha intrapreso un accurato studio di zoologia medica, ricercando le zanzare in tutta l' Italia, sia nei paesi di malaria, sia nei paesi immuni; ed ha dimostrato che nei paesi di malaria vivono particolari specie di zanzare le quali mancano nei paesi immuni."

In any case, even if it be admitted—and I do not admit it—that I was mistaken, it is absolutely certain that my mistake would have led me to the discovery of the truth: and Prof. Nuttall will not be unaware that this would not have been the first time that such a thing had happened in the history of biology.

(5) It is by no means proved that, in India, there are no other malarial parasites of mammals or birds capable of developing in *Anopheles*. Ross himself, in the *Report of the Malaria Expedition* (1900), writes that the parasites found in bats and monkeys "may perhaps be identical with the human species....Exhaustive search in the blood of other mammals besides bats and monkeys is still required. In many places dogs are popularly supposed to get malarial fever" (p. 32). In face of such uncertainties, which, to a considerable extent, have not been cleared up even at the present day, nobody can assert without a shadow of doubt that the "pigmented cells" observed by Ross were "the early stages of the development of one species of human malaria parasite (*praecox*) in *Anopheles*" (*Parasitology*, 1924).

(6) Prof. Nuttall writes: "Ross subsequently discarded this experiment." But what he attributes here to Ross was actually done by Grassi. On consulting the letter from Charles to Ross, mentioned above, we find the following passage: "You say in your letter that possibly tertian may develop in your grey mosquito, Culex pipiens. Do let me ask you to turn early attention to this point. I know you have published one case. It seemed, possibly, to support this idea. With proteosoma about, of course, this single observation is otherwise explicable. The Culex pipiens is one of those mosquitoes widely diffused in places where there is no malaria, therefore the chances are immeasurably against its being a host for tertian....Only yesterday, one of the workers for the first time made an observation which showed me that he had spotted this weak point in your armour." (The worker referred to was myself.)

(7) Prof. Nuttall writes: "It seems certain that Grassi was after all entirely guided by Ross's publication of December 18th, 1897, in which he describes an insect with spotted wings, and eggs like those which characterise *Anopheles*." On the contrary, it not only seems but is absolutely certain that the data supplied by Ross regarding his mosquitoes with spotted wings gave me no guidance whatsoever. In support of this assertion I will also once more repeat the evidence of Charles, whom Ross uses as a witness against me. Charles testifies in a letter to me that he made my acquaintance *after* November 4th, 1898, and that he gave me the numbers of the *British Medical Journal* (18. xii. 1897 and 26. ii. 1898) containing Ross's articles, which I had not seen before.

Moreover, Eckstein, the only entomologist who has seriously studied the history of the discovery of the malaria-bearing mosquito, has fully appreciated the absurdity of supposing that I could have arrived at the discovery of *Anopheles* from the data furnished by Ross. In 1898 *Culex* was distinguished from *Anopheles* by the length of its palps, of which Ross makes no mention, and its ova were entirely unknown before I studied them. With all respect to Prof. Nuttall I must repeat that it is absurd to maintain that an entomologist, who had never made a special study of Diptera, after reading in Italy that certain mosquitoes in India had spotted wings and boat-shaped eggs, could divine that they were *Anopheles*—more especially as the term "mosquito" is very vague. "Many different insects, belonging to the genera *Culex*, *Anopheles*, *Aedes*, *Ceratopogon*, *Simulium*, and *Phlebotomus*, are called Mosquitoes" (Nuttall, *Centralbl. f. Bakt.* p. 292, 1899).

(8) In *Parasitology* Prof. Nuttall now writes: "There can be no question but that Ross observed the early stages of the development of one species of human malaria parasite (*praecox*) in *Anopheles*. Unfortunately, for reasons which are fully explained in his *Memoirs*, Ross was unable to do more than to predict that the immature forms ('pigmented cells,' 1897) observed by him in *Anopheles* would complete their development as in *Proteosoma*." But this, instead of the conclusions discussed above, is surely what Ross himself should have written in summing up the results of his work in India—*not Nuttall, a quarter of a century afterwards*. Contrary to Prof.

Nuttall's belief, also, it is evident that Ross himself, after failing in India to infect *Anopheles* in accordance with the indications supplied to him by Charles, must have been still in doubt as to whether his "dappled-winged mosquitoes" really were *Anopheles*, and consequently in the French summary of his work he spoke only of "two mosquitoes of a new species."

But one cannot ignore the fact that a parasite may often begin to develop in one or other of its supposed hosts and then go no further. It is always difficult, therefore, to prophesy what is going to happen...unless, as in the present case, the prophet practises his profession twenty-five years after the event.

THE BIONOMICS OF *IXODES HOLOCYCLUS* NEUMANN, WITH A REDESCRIPTION OF THE ADULT AND NYMPHAL STAGES AND A DESCRIPTION OF THE LARVAE.

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(With Plate XV and 2 Text-figures.)

Ixodes holocyclus is a tick of considerable economic importance not only in New South Wales but along almost the whole coastal region of Eastern Australia, where it causes an extremely common and fatal paralysis of dogs and cats. This paralysis, while especially prevalent in the spring and early summer, may occur at any time of the year. Young foals, calves and lambs are also said to be affected, while more important still, authenticated cases are not lacking of children experiencing serious and even fatal attacks of tick paralysis.

As a preliminary to carrying out research into the nature of the causative agent of tick paralysis and the curative and prophylactic measures to be adopted, the following work was undertaken to ascertain something of the life cycle of this parasite.

[A redescription of the adult and nymphal stages appears desirable, with a description of the larvae, as Neumann's original description of the adults and nymphs requires considerable alteration.]

At the time of the inception of this work, practically nothing was known of the biology of this species, though it had first been experimentally proved to be one causal agent of tick paralysis by Dr S. Dodd (1921) of the Veterinary Pathological Department, University of Sydney, while Professor L. H. Harrison had specifically identified the ticks used by a former worker.

Method of obtaining material. At the time this work was commenced, late in the summer, there was considerable difficulty in obtaining living ticks with which to commence operations. Dogs running in the bush would only rarely be found with ticks on them, and these even if detached generally died very shortly (Dodd). *Ixodes holocyclus*, even when most prevalent, will rarely be found more than four or five at a time on domestic animals, unlike other species of ticks such as *Haemaphysalis bispinosa* or *Boophilus australis* which may be present in all stages in enormous numbers. Since Neumann has recorded it from various Australian animals, it appeared probable that the dog was not the natural host, in that it is so frequently killed by the tick during the process of engorgement that it would be unsuitable for the propagation of the species.

For these reasons an effort was made to secure any small native mammals from around Sydney in the hope that these would prove to be a source of supply. Through the kindness of Mr D. Selkirk of Killara, I was enabled to secure three specimens of the bandicoot (*Perameles nasuta*), a small rat-like marsupial commonly found in the scrub round the foreshores of Sydney Harbour, and from these a supply of gorged larvae and nymphs and a few unfed adults were obtained.

Method of Collection.

The bandicoots were caught in a box trap, which did not injure them in any way, and then transferred to a cage with wire netting floor standing in a shallow tray of water, after the method of Brumpt (*vide* Langeron, 1921). Each morning and evening the tray was examined and large numbers of gorged larvae and nymphs were in this way obtained. While many of the larvae and nymphs would be found floating in the morning, great numbers were found under water on the bottom of the tray. These however seemed to stand the long immersion well, though the subsequent mortality amongst them appeared to be heavier than amongst those floating. It was noticed that very few larvae or nymphs dropped off during the day, practically all those found being recovered in the morning having left the host during the night, when the bandicoots are active. The larvae and nymphs when taken out of the water were dried on blotting paper and then transferred in small lots to tubes with blotting paper at the bottom. Slips of cardboard on blotting paper were also placed in the tubes, to enable them to climb easily, and the tubes were loosely stoppered with cotton wool.

No gorged adults were ever recovered from any bandicoots, though three unfed females were collected from an injured bandicoot which was chloroformed. This peculiarity will be touched on later.

From the gorged larvae and nymphs so obtained the life-cycle of *Ixodes holocyclus* was ascertained. Various lots of larvae and nymphs were kept under varying conditions of heat and moisture, while experimental animals used for the unfed stages to gorge upon consisted of bandicoots, guinea-pigs, dogs, goats, and last but not least, man. All work was carried out at the Veterinary School, University of Sydney, with material thus obtained.

Ixodes holocyclus was first described and figured by Neumann (1899), in the adult and nymphal stages, no mention being made of the larva. Nuttall and Warburton (1911) reprint Neumann's description, with one or two alterations. Neumann's description of the adults is based on: 2 ♀ and 1 ♂ from Australian sheep, 1 ♀ from *Phascogale penicillata* (Marsupial tree-shrew) from Port Stephens, Australia, 1 gorged ♀ from dog in Queensland (Bureau of Animal Industry, Washington), 2 gorged ♀ of unknown origin (Hamburg Museum), 1 ♀ unfed from New South Wales.

His description of the nymph is based on one specimen from *Sciurus variabilis* in India (British Museum), and from East India (Berlin Museum),

one microscopic specimen from red squirrel from India (R. Blanchard, coll.).

It will be seen that Neumann had only been able to examine one ♂ and a limited number of ♀♀, and as there are one or two points that need rectification, re-description is desirable. Neumann's description of the nymph does not agree with that of the numerous specimens I have examined, which is not surprising as none of his specimens came from Australia. The larva has not previously been described.

REDESCRIPTION OF *IXODES HOLOCYCLUS*.

Male. (Pl. XV, figs. 1–4.)

Body oval, narrowed in front, broadly rounded posteriorly. Size: varies considerably, three specimens without capitulum being as follows, 2·7 mm. × 2 mm., 2·6 × 2 mm., 1·9 × 1·6 mm. (Neumann gives the size *with* capitulum as 3 × 2 mm.). Yellowish brown in colour in living subjects, showing through the scutum, in old specimens, the black intestinal caeca.

Dorsum (Pl. XV, fig. 1). Scutum (size 2·4 × 1·5 mm., 2·2 × 1·4 mm., 1·7 × 1·2 mm.), glossy, convex with numerous very fine punctations on anterior third, less numerous posteriorly. In some specimens there is a clear outline of a pseudo-scutum. (Neumann states that there are no punctations on the scutum.) Scapulae blunt. Cervical grooves short, and continuous in front with the lateral grooves. Marginal fold narrow with numerous fine punctations. Few very fine hairs on marginal fold, very few or lacking on scutum.

Venter. Convex posteriorly in newly hatched subjects, concave throughout in old specimens, sparsely covered with fine hairs. Pregenital plate hexagonal, broader than long. Genital orifice broad, opposite 2nd inter-coxal space. Anal plate may be flattened anteriorly or convex and rounded, lateral borders convex, meeting or not quite meeting at a point behind. Median plate longer than broad, very broad behind, lateral borders diverging sharply and then less sharply, forming an angle between upper and lower portion. Adanal plates in the form of oblique parallelograms. Spiracles elongated, tapering somewhat behind, vary considerably in shape from less than twice as long, to more than twice as long as broad, whitish in colour, macula anterior of middle.

Capitulum (Pl. XV, figs. 2 and 3). Short (0·5–0·6 mm.) base quadrangular, broader than long, shows two areas of punctations dorsally suggesting the porose areas of the female.

Text-fig. 1. Digit of ♂.

Digit (Text-fig. 1). Length 130 μ. (Neumann gives length as 160 μ.) Internal article, terminates in three fine serrations, no dorsal process. External article with four teeth, anterior two being very small.

Palps short and broad, first article prominent, second and third articles fused. Article 4, very small, subterminal. Internal border strongly convex, external almost straight.

Hypostome slightly emarginate. Five or six small denticles, then 2/2 files. Teeth increasing in size to fifth row, which is much blunter than fourth, sixth row represented by a raised ridge, then perhaps faint indications of further rows.

Legs. First and fourth legs reddish, with barred appearance, second and third legs paler in colour, yellowish. Coxae contiguous, with external spur, that of coxa I being the largest, the others sub-equal or diminishing a little from I–IV. Long hairs on posterior border of coxae. Tarsus I somewhat humped, terminating obliquely. Tarsus IV nearly four times as long as broad, tapers dorsally, pseudo-articulation slightly proximal to middle. (Pl. XV, fig. 4.)

Female. (Unfed.) (Pl. XV, figs. 5–10.)

Body, flat, oval, broadest posteriorly. Dull greyish yellow in living subjects (splotched with black of the intestinal caeca).

Size. Varies considerably even in individuals obtained from the same lot of nymphs. Four individuals measured (without capitulum) 3·5 × 2·5 mm., 3·3 × 2·6 mm., 3·4 × 2·5 mm., 2·6 × 1·11 mm. (Neumann gives the size *with* capitulum as 3·2 × 1·7 mm. This would make the female smaller than the male, which Neumann states is 3 mm. long *without* capitulum, which would be very unusual.) When fully gorged, the body may be grey-green, or greenish black in colour, broadest behind, with white lines radiating from spiracles; contracted on a line with the spiracles. Largest specimen I have collected was 12 mm. long. (Neumann gives largest as 15 × 11 × 9 mm.) Complete marginal fold of equal width throughout. Few fine hairs on marginal fold, fine hairs sparsely scattered over dorsum.

Scutum (Pl. XV, fig. 5). Reddish yellow in preserved specimens. In living specimens, yellow in the median field, with dark brown lateral areas. Slightly broader than long, or as broad as long (2·1 × 2 mm., 2 × 2 mm., 2·1 × 2·1 mm., 1·6 × 1·55 mm. Neumann says longer than broad 2·3 × 2 mm.). Broadest posterior to middle. Flat medially, with lateral borders marked off by lateral grooves, raised and convex. Cervical grooves, convergent, short and superficial. Lateral grooves well marked attaining posterior border, accentuated by an external ridge. Punctations very numerous, subequal, confluent in places.

Venter (Pl. XV, fig. 6). Vulva at level of third intercoxal space. Sexual grooves divergent, then tending to curve inwards behind the level of the anus. Anus posterior, situated in an elongated depression. Anal grooves convex anteriorly, convex laterally, and curving towards each other to meet at a point on the median line. (Anal grooves may be more or less straight and parallel laterally and flattened anteriorly.)

Spiracle. Small, circular, reddish, considerably posterior to coxa IV. Its position with regard to the middle, noted by Neumann, varies according to the state of engorgement.

Capitulum (Pl. XV, figs. 7, 8, 9). L. 1–1·2 mm. (Neumann gives 1·3 mm.) Reddish yellow. Base pentagonal. Posterior border undulating, with slight or negligible cornua, broader than long, lateral borders sub-parallel then converging anteriorly. Porose areas deep, sub-circular, may be slightly pointed postero-externally. Auriculae forming prominent, blunt, retrograde processes. Hypostome long, narrow, lanceolate. Numerous small denticles, then 3/3 files of 12–13 teeth, increasing in size from within outward. Convex ventrally with inner rows of teeth superimposed on the outer. Digit 190 μ long. (Neumann gives 210 μ.) Dorsal process, with two retrograde teeth, prolonged by its shaft of insertion down to lower third of digit. External process with five teeth of increasing size, anterior two being very small. Palps long (Nuttall and Warburton repeat Neumann's statement that the palps are much longer than the hypostome, this does not appear justified in any specimens I have seen. (See figs.) Article 1 salient outwards near base. Articles 2 and 3 not very distinctly separated, article 2 twice as long as article 3, article 4 very small and sub-terminal. Whole palp cultriform, convex dorsally, concave towards inner edge. Concave ventrally, with convex outer edge.

Legs reddish, in some specimens there is distinct banded appearance due to lighter colouring of the distal extremity of the various articles of the legs. Legs ii and iii are lighter in colour than i and iv, and yellowish. Coxae large, trapezoid, contiguous, with external spur, much stronger in coxa I, and decreasing in size from coxae I–IV. (Neumann states that the coxae are separated by intervals almost equal to their width. While this is so in gorged females, his description is supposed to apply to the unfed female.) Long white hairs on posterior border. Tarsus I (Pl. XV, fig. 10) somewhat humped, tapering obliquely. Tarsus IV almost four times as long as broad, abruptly tapering, pseudo-articulation nearer proximal extremity of the article; claws short, the pad almost reaching to their tips.

Nymph. (Pl. XV, figs. 11–16.) Differs from female as follows:

Body oval, 1·2 × 0·9 mm. (without capitulum). Broadest a little behind coxa IV. Gorged nymph 3·5 × 2·5 mm. Scutum 0·7 × 0·7 mm.

Spiracles situated very posteriorly, almost on a line with the anus. Anal grooves do not meet at a point behind, and are not continuous anteriorly.

Sexual orifice lacking, represented by an elongated plate (*anlage*), extending from 1st to 3rd intercoxal space.

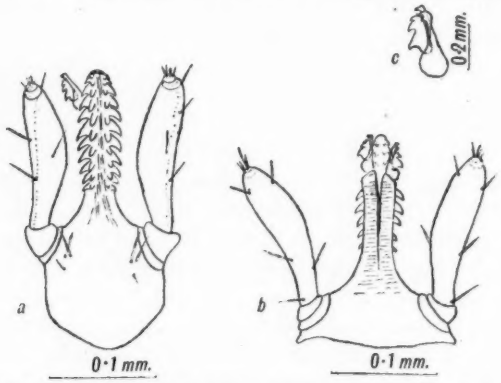
Capitulum 0·4 mm. Digit (Pl. XV, fig. 14) 70 μ long, very similar to that of the female. External article with four instead of five teeth. Hypostome (Pl. XV, fig. 13) with 3/3 and then 2/2 rows of 11–12 teeth, of which the external are sharp and the inner blunt.

Palps without apparent articulation between articles 2 and 3. Coxal spurs less marked.

Larva. (Pl. XV, figs. 17 and 18.) Differs from nymph as follows:

Body oval 0·5 × 0·4 mm. Broadest just behind coxa III. Gorged larva 1·1 × 0·9 mm. Scutum 0·3 mm. long. Broadest anterior to middle. Cervical

grooves reach posterior border. Anal grooves do not converge behind, more incomplete anteriorly. Capitulum (Text-fig. 2) 0·2 mm. long dorsally. Digit 0·40 μ long, very closely resembling that of the nymph. Hypostome with 2/2 files of 10–11 teeth, inner row blunt, external sharp. Coxal spurs very minute.



Text-fig. 2. (*a*) Capitulum of larva, ventral view; (*b*) dorsal view; (, digit of larva, dorsal view.

Eggs 0·54 × 0·4 mm. Oval. Pale yellow when freshly laid, then darkening considerably.

The above description is based on a large number of adults both male and female. The males in all cases were raised in the laboratory from gorged nymphs and larvae recovered from *Perameles nasuta* (the bandicoot). The females were in many cases raised in the laboratory in the same way as the males, but also included a number of specimens obtained from dogs and cats, and man, while one gorged female was from *Trichosurus vulpi* the large bush opossum. All the nymphs and larvae were with one exception collected from bandicoots in the laboratory. One nymph was from *Rattus rattus* (the so-called bush rat, but not really an indigenous species).

Biology.

1. *Life-cycle of* Ixodes holocyclus.

Like all other known species of Ixodes whose biology is known, *Ixodes holocyclus* is a three-host tick, gorging and then dropping off the host between each successive stage of the life cycle. Large numbers of larvae, nymphs, and adults (females) may be found on the same individual.

2. *Times of engorgement of larvae:*

From the few batches of fertile eggs which I was able to hatch out in the laboratory large numbers of larvae were obtained, and these were placed on guinea-pigs, but in no case were any gorged larvae recovered. This is peculiar in that both nymphs and adults engorged on guinea-pigs.

From the period over which gorged larvae were recovered from bandicoots

captured in the bush we are enabled to judge the period taken for larval engorgement.

Larvae recovered on day.

	1st day after capture	2nd	3rd	4th	5th	6th	7th
Bandicoot *A*	20	8	105	62	26	12	—
" *B*	146	84	302	165	6	—	—
" *C*	28	20	Not recorded	Not recorded	15	—	—

Presumably the animals were picking up unfed larvae up to the night of their capture. In the case of bandicoots *A* and *B* there is a marked drop in the number of larvae recovered after the fourth day from that of their capture. This may probably be interpreted as an indication that larvae commonly take four days to engorge, but in *A*, *B*, and *C* numbers took five days, and in the case of *A*, some took six days, but none seven. We may therefore assume that larval engorgement usually takes from 4–5 days and may take as long as six days.

From an injured bandicoot which was chloroformed several larvae were obtained, three of which attached themselves to the arm of Mr A. R. Carne and remained attached for three days, during which time they increased in size but were not apparently fully gorged. They were then rubbed, or dropped off.

Some 25 larvae, reared in the laboratory were liberated on my arm, and 12 were later seen to have fixed themselves. Some of these remained attached for two days, but owing to the intense irritation they set up were either scratched off or rubbed off during the night. They had up to that time shown little increase in size.

3. *Time between gorged larvae dropping off host and emergence of nymph.*

It was found that the date of the first moult was very greatly influenced by the degree of heat and moisture at which the gorged larvae were maintained. The optimum temperature (that at which the earliest moults occurred) was found to be about 27·5° C. (80° F.) under suitable conditions of moisture, when the quiescent period was about 20 days. At room temperature it was extended to about 40 days.

It would appear likely that the greater cold experienced under natural conditions in mid-winter would prolong this period very greatly but probably would not prevent the ultimate metamorphosis, since in some small lots kept at room temperature 100 % moulted.

4. *Period between first moult and seeking host.*

It was found that nymphs on just emerging showed very little tendency to move about for two or three days, but would climb gradually up the tube and walk about slowly if exposed to bright light. In four days the majority were clustered about the top of the tube, and in five or six days would become active on being disturbed. It is probable that at this stage nymphs would fix themselves to any host that offered itself. Nymphs attached themselves and engorged on being placed on a bandicoot eight days after moulting.

5. *Period of engorgement of nymph.*

The period in which the nymph remains attached to the host has been ascertained as from 4–7 days, that most frequently found being 5 days. This has been found to be so both from the recovery of gorged nymphs from bandicoots and from placing unfed nymphs on a variety of experimental animals.

(*a*) Gorged nymphs recovered from bandicoots on the days succeeding capture:

	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Bandicoot 1	14	2	10	14	10	—	—
„ 2	5	11	8	5	—	—	—
„ 3	9	7	Not recorded	Not recorded	3	—	—

It will be seen that no nymphs were recovered later than five days after the bandicoots were captured.

(*b*) Unfed nymphs placed on bandicoots:

	No. placed on bandicoot	Gorged nymphs recovered on 3rd day	4th day	5th day	6th day	7th day
Lot iv	20	—	3	11	—	—
„ v	—	—	1	4	—	—
„ vi	14	—	—	7	2	—
„ vii	14	—	—	5	1	—
„ viii	22	—	1	12	0	1

(*c*) Nymphs on guinea pigs and dogs:

	No. of nymphs	Gorged nymphs recovered on 3rd day	4th day	5th day	6th day	7th day
Lot ix (guinea pig)	8	—	—	1	1	—
Lot x (dog)	8	—	4	2	—	—

These nymphs recovered from the dog were slightly smaller than those obtained from the bandicoots, but subsequently metamorphosed.

(*d*) Nymphs on man.

Out of several nymphs placed on my arm, two fixed themselves, one however dropped off in a few hours. The other remained attached for three days when it dropped off. It then appeared almost fully gorged but was smaller than those obtained from bandicoots and died almost immediately.

It will be seen nymphs most frequently take five days to engorge but may take as little as four or as much as seven days.

6. *Period between engorged nymph leaving host and emergence of adults.*

It was noticed that both larvae and nymphs show signs of activity for as much as a week after their dropping off a host, and will climb up the tube if disturbed. A large number like to attach themselves to the wool stoppering the tube, where they will remain if undisturbed during this period of quiescence. After a varying time, according to the temperature, the nymphal skin begins to show a light area anteriorly and this increases gradually as the time for the second moult becomes more imminent. Healthy specimens can easily be

distinguished from those that have died as the old nymphal skin changes to a light brown, while the clear area, due to the separation of the growing adult from the old skin, becomes more noticeable. Several days before the moult occurs, when specimens are cleared in lactophenol, the form of the adult can easily be seen with its legs enclosed in the legs of the nymphal skin.

As with larvae, cold was found to lengthen the time taken before the moult, but did not prevent its taking place. The shortest period taken was that at which nymphs were incubated at a temperature fluctuating between 24–27° C., the first adult emerging after 20 days. At room temperature falling as low as 10° C., where nymphs were kept under moist conditions, adults emerged in from 53–65 days. Dry atmosphere was either rapidly fatal, as in most cases, or prolonged the period somewhat (up to 71 days at room temperature) above that of other lots.

Lot	Temperature	First moult	Last moult
i	Room temperature of 16–20° C. for ten days and then incubated at ca. 27° C.	23	29
ii	" " " "	25	26
iii	Incubated 24° C.	21	23
iv	" 24–27° C.	20	Not recorded
v	" 20–22° C.	25	28
vi	Room temp. 19–26° C.	32	37
vii	Room temp. of 16–20° for three weeks then fell to 10–14° C.	53	65

In all the lots producing both males and females the first adults to emerge have been males. In some cases all the males in the lot would emerge before any females.

7. *Period between emergence of adults and seeking of final host.*

Males when they first emerge are shiny black, with rounded bodies, slightly convex on both dorsal and ventral surfaces. After one or two days they tend to become lighter in colour due to the passage of large quantities of black faecal material. In a few days they begin to appear yellowish-brown in colour, while their ventral surface becomes less convex. The legs at the same time change from a pale yellow to reddish. As time goes on the ventral surface becomes markedly concave and the scutum transparent, so that the outline of the black intestinal caeca can be clearly seen.

The females when first emerging are much lighter in colour than the males, having a semi-transparent greyish appearance, the body being thick and rounded posteriorly and somewhat convex on both dorsal and ventral surfaces. The legs at first are very pale, almost colourless and transparent. The body darkens rapidly in the first day or two and becomes a dull greyish yellow while the scutum is yellow in the median field with dark lateral borders.

For the first two or three days after moulting there is little movement or activity; then they commence to move up tube and in six or seven days become quite active if disturbed. Females placed on dogs 9 days after hatching attached themselves and engorged. In winter females which are at room

temperature are much less active when disturbed than those kept in incubator, but commence to move about rapidly if warmed or placed on a host.

8. *Coupling.*

The term coupling is used as suggested by Nuttall and Merriman (1911) to indicate that period during which the male and female remain attached to enable copulation to take place. Coupling apparently occurs away from the host, as I have never found males and females coupled on either wild or domesticated animals. Males are occasionally found on the host however, though it is probable that their presence there is accidental and temporary.

Males and females reared under laboratory conditions readily couple in the tubes in which they have emerged. The usual time for females is about five days after emerging, that is as soon as they have hardened and commenced to become active. Males however will couple with females that have moulted only 24 hours previously.

One male may couple with more than one female, and a female may recouple with different males. Coupling was observed on occasion as long as 35 days after moulting, even though the contracting parties had been continuously associated during that period.

The duration of coupling varies very considerably, from a few hours to three or four days. One pair kept at room temperature of 20–25° C. remained coupled for as long as 11 days.

Coupling in *Ixodes holocyclus*, in general appears to resemble that described for other species of the Ixodidae. When a pair are coupled the hypostome of the male is deeply buried in the genital orifice of the female while the palps are widely abducted on either side. Although coupled pairs have been watched for considerable periods I have not been fortunate enough to observe the phenomena of fertilisation described by Samson (1909), Nuttall, and Lewis (1900) for other species of ticks.

I am not certain whether females require to be fertilised before engorging but am inclined to the opinion that this is not necessary. In three cases of fully gorged females recovered from wild and experimental animals, oviposition has taken place normally but no eggs hatched, while in the case of a gorged female known to have been fertilised, a high percentage of eggs hatched. Samson (1909) states that *Ixodes ricinus* will only attach itself to a host during or after copulation, but Nuttall considers that this requires confirmation. In the case of one female, known not to have been fertilised, although it attached itself to a dog, it died without engorging; this tick however came from a sickly batch, others of which had died before being placed on a host. Other females also have been known to die on hosts without engorging but I am not certain that they had not been fertilised.

9. *Period of engorgement of female and sojourn of male on host.*

The site of attachment of females on dogs is more particularly round the anterior part of the body, especially behind the ears, on the jaws, and on the

neck. It was found that females that had been without food for long periods were less particular about selecting favourite positions, and if placed on the head or neck would often immediately settle in position. Females however that had not long hatched would often wander about for long periods before attaching themselves. I have been unable to get them to anchor on the under surface of the body or on the legs of experimental dogs, but one attached itself on the axilla. For experimental work an easy place to get them to attach themselves was inside the ear and this had the advantage that dogs were unable to scratch them off. I have heard of one case of a female anchoring between the toes of the fore-paw.

I have never succeeded in getting males to attach themselves to experimental animals nor have I found them on animals running in the bush. If placed on bandicoots, dogs or guinea-pigs they were usually recovered a few hours after, never showing any sign of engorgement. On one occasion however a male that had escaped from a tube was found attached to the finger of a student, and when forcibly detached a small drop of blood issued from the site of attachment.

Once a female has decided to attach itself, the long hypostome (over 1 mm.) is inserted with remarkable rapidity, and in little more than one minute will be deeply buried. No pain is caused apparently during the process as no animals have ever shown any discomfort, beyond in one case a slight twitching of the skin. People who have picked up ticks in the bush never seem to feel their presence, while they are attaching themselves.

Females do not always engorge after attachment and may die in position without engorging appreciably even though they have not been injured. They have never been seen to change their position when once attached, except on the death of the host. Three adults, obtained from a dead bandicoot, on which they had probably fed slightly, reattached themselves and engorged when placed on guinea-pigs.

The period occupied in engorgement varies very greatly according to temperature at which the experimental animal is maintained. Adults have remained attached for as long as 21 days in winter weather during which time the temperature fell as low as 6° C. during the night. The shortest period taken by unfed ferrets was six days in warm summer weather.

Though females were frequently placed on bandicoots, which are apparently among the natural hosts, no gorged females were ever recovered from them. It is possible that they may have fallen off into the bags in which the bandicoots slept during the day and that they had been eaten, since bandicoots will eat raw meat, grubs and insects. One recovered female was damaged as if it had been scratched off.

Females were repeatedly placed on my arm and legs in an endeavour to get them to engorge but without success, even though they are frequently picked up in the bush, and engorge on man.

The process of engorgement may be said in general to follow fairly fixed

and definite rules and may be described as follows. For the first 24 hours there is little if any increase in size, but there may be a slight dorso-ventral thickening, of the posterior part of the body. On the second day this thickening becomes more pronounced and there may be a slight increase in length apparent. This gradual increase in size continues on the third day, the body now being greyish in colour and noticeably lengthened; on the fourth day there is again a noticeable increase in length, but little increase in thickness. On the fifth day the tick will be seen to be considerably increased in length and breadth compared with the previous day and to have become darker in colour. The period of final rapid engorgement is now being entered upon, and the following (6th) day the tick is found to have increased enormously not only in length but also in thickness, to have become glossy greenish or bluish black; it completes engorgement and drops off in a few hours. So definite is this process that in warm weather when engorgement is rapid one is enabled to tell to a few hours when each stage in the process will be reached by the appearance of the tick, and this is of practical importance in experimental work on tick paralysis, the commencement of symptoms coinciding with the reaching of a definite stage of engorgement.

Protocol of time taken in engorgement in a number of cases:

Animal used as host	Tick	Climatic conditions at time	Days taken in engorgement
Guinea-pig	No. 1*	Room temperature 16–20°	5
,,	No. 2*	,, ,,	6
Dog	No. 3	Warm (shade temp. up to 24° C. at mid-day)	6
,,	No. 4	,, ,, ,,	6
,,	No. 5	Hot (shade temp. often over 27° C. at mid-day)	6
,,	No. 6	Mid-winter temp. falling at night to 6° C.	21
,,	No. 7	,, ,, ,,	17†
,,	No. 8	,, ,, ,,	11‡
Goat	No. 9	Weather warm	7
,,	No. 10	,, hot	8

* Found on dead bandicoot, may have already fed a little.

† Dog died of paralysis. Tick dropped off not fully gorged.

‡ Dog developed tick paralysis and tick taken off partly gorged.

From the above it would appear that the sixth day is in general the earliest on which engorgement is completed, while the cold of winter may prolong the time taken up to 21 days.

10. *Pre-oviposition period.* (*Between engorging of female and commencement of oviposition.*)

After females have dropped off the host, they will for some time exhibit signs of considerable activity. Thus under natural conditions they will walk considerable distances, so that when they drop off experimental animals in pens or kennels, they may be lost unless careful watch is kept during the final stage of engorgement. When placed in tubes they will climb to the top of the tube and move about actively if disturbed. After some days, however, they become less active and disinclined to move, and exhibit a desire to take cover, wedging themselves in between the paper and the side of the tube.

11. *Oviposition.*

On the very few occasions on which oviposition has been observed it has, as might be expected, been seen to resemble in general the process as described for other species and genera. One point noticed, however, was that Géné's organ might be protruded and retracted alternately for a long period of time without oviposition occurring. The mouth-parts were often markedly lowered to lie in proximity to the ventral surface of the body, coinciding with this protrusion of Géné's organ, but even then oviposition might not occur. Géné's organ appeared as a glistening, irregularly lobed bladder-like structure, there being small lobes projecting from the main mass. It was noticed that during the period over which oviposition took place rhythmical waves were observed through the ventral body wall to pass posteriorly from the region of the genital orifice about every six seconds. What relation this bears to oviposition is not known. Just before oviposition the mouth-parts were lowered to lie along the ventral body wall, the tips of the palps and hypostome reaching almost to the genital orifice.

For a few seconds the palps lie close to the hypostome, then immediately prior to oviposition they are widely abducted, while at the same time the ovipositor is extruded carrying the egg, which is placed well up the hypostome, towards the basis capituli, and seized by Géné's organ. The palps and hypostome are then elevated dorsally so that the newly laid egg comes to lie on the dorsum of the body. It appeared that the ovipositor placed the egg much further along the hypostome before it was seized by Géné's organ than is shown in Wheler's figures. Géné's organ also appeared only to cover the egg while the palps were brought together and the mouth-parts raised. This may have been due however to disturbing the female in an effort to see the whole process.

The period elapsing between the laying of successive eggs was not taken but since 200 eggs may be laid during a day, one egg must be laid at least on an average about every six minutes. The period over which oviposition takes place is subject to considerable variation, ranging from a minimum of 16 to a maximum of 34 days. The number of eggs laid per day may reach a maximum of 200 during the early period of oviposition, while towards the latter end only 20 or less a day may be laid. Females die as a rule within a day or two of the cessation of oviposition. The number of eggs laid varies from about 1800–2500.

Tick	Temp. kept at	Day after dropping off host at which oviposit commenced	Duration of oviposit	Eggs laid
No. 1	24–27° C.	11th	16 days	1770
No. 2	20–22° C.	12th	19 „	2400
No. 3	Room temp. ca. 18–20° for 21 days then 21–23·5° C.	20th	34 „	2460

The following table is given to show the number of eggs laid per day during the whole period of oviposition in the case of tick No. 1.

Tick No. 1:

Day after oviposition commenced	1–2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Approx. no. of eggs laid	200	200	200	150	130	120	180	150	120	100	80	40	40	40	20

The tick died on the 17th day after oviposition commenced.

In the case of tick No. 3 very few eggs were laid during the last six days, the daily totals from the 28th to 34th day being 3, 17, 36, 27, 13, 5, 3.

12. *Time taken between oviposition and hatching of eggs.*

The first eggs hatched in lot i (laid 8. x. 23) in 49 days. First in lot ii (12. x. 23), in 61 days. Both lots were kept in a saturated atmosphere.

13. *Effect of heat and moisture upon life-cycle.*

It has been found under experimental conditions that a dry atmosphere is rapidly fatal to both eggs, gorged larvae, and nymphs, while unfed adults which have been raised in the laboratory seem to be not quite so susceptible. Eggs, unless kept in a suitable degree of moisture, rapidly darken in colour and collapse. Exposure to direct sunlight even for a short period also seems to affect them in a very adverse manner. To ensure that eggs did not dry up, the wool stoppering their tubes was thoroughly saturated every second day with water, while the blotting paper on which the eggs were lying was also moistened from time to time.

It was found that when gorged larvae were kept dry, even at moderate room temperature of 16–20° C., the majority appeared to be dead in 1–2 days. The larvae were kept in glass tubes, lightly stoppered with cotton wool, the wool being removed and the air changed at least once a day.

Gorged nymphs also appeared to be almost as adversely affected by dry conditions for the great majority appeared to be dead or unhealthy in four days. Very few nymphs kept under these conditions metamorphosed.

Dodd found that adults taken in the bush died very soon when placed in boxes or bottles, so that he experienced considerable difficulty in getting them to his laboratory alive. It is probable that the influence of heat and dryness account for the seasonal incidence of the various stages under natural conditions. Thus adults are most numerous in the suburbs of Sydney during the spring and early summer months. This is, I think, due to the fact that the various metamorphoses and hatching of eggs are greatly prolonged during the cold winter months, while the warmer spring and early summer weather, with heavy dew at night, make a suitable environment for development, with the resulting rapid metamorphosis and abundant occurrence of the adult stage. Then as the summer advances and conditions become hotter and dryer, ticks are found to be less numerous, so that on a bandicoot captured on December 12th, no larvae, nymphs, or adults were obtained, the hot dry weather of November probably being fatal to large numbers of gorged nymphs and larvae. Adults are affected to a less degree so that cases of tick paralysis caused by the adult female continue to be common through November and December. Then, with autumn rains and cooler weather, conditions are again rendered more favourable to development, so that on bandicoots taken in the first week of May, great numbers of larvae, nymphs, and some adults were obtained. During the later winter months (August) some larvae and

nymphs may be found on bandicoots, occasional adults also being met with.

14. *Longevity of the unfed tick (under most favourable conditions).*

(*a*) *The unfed larva.* The longest period over which larvae have remained alive unfed is 44 days, while in another lot none were alive after 33 days. From these two lots, larvae placed on guinea-pigs 16 or 23 days respectively after hatching, no gorged larvae were recovered. It is possible therefore that the larvae were in a sickly condition, their vitality being too low to permit of engorgement.

(*b*) *Unfed nymphs.* Few nymphs survive unfed for more than 31 days when kept under favourable conditions.

It would appear that both larvae and nymphs are only able to survive for a comparatively short period of time, this is in marked contrast to the longevity of unfed larvae and nymphs of *Ixodes ricinus*.

(*c*) *The adult.* Adults both ♂ and ♀ have been kept alive for considerably longer periods than the immature stages but unfortunately I have been unable to estimate the maximum period for ♀♀, as all those available were used in experimental work before the maximum period had elapsed. Females have, however, been kept unfed for 77 days and then engorged on being placed on a host. Males have survived for 142 days.

The comparatively short period during which larvae and nymphs of *Ixodes holocyclus* may remain alive unfed, together with the high mortality rate of the gorged stages under unfavourable conditions may perhaps explain why the numbers of this species found on domesticated animals are never very great, compared with that of another scrub tick, *Haemaphysalis bispinosa*, which is often found in enormous numbers on one animal.

15. *Time required to complete life-cycle.*

The foregoing observations enabled us to estimate the time taken for a generation to develop on warm-blooded hosts. It is clear that the time taken may be very much prolonged under unfavourable conditions, such as cold, lack of host, etc., so that in nature it is possible that development may take a year or more.

	Days required	
	Shortest period	Longest period
Fecundated ♀ abandons the host, begins to oviposit ...	11	20
Larvae hatch out after	49	61
Larvae harden and wait for host No. 1	7	44
Larvae remain upon host	4	6
Nymphs issue from larval skin	19	41
Nymphs harden and wait to attack host No. 2	7	31
Nymphs remain on host No. 2	4	7
Adults issue from nymphal skin after	21	71
Adults harden and await host No. 3	7	77
Adults remain on host	6	21
	135 days	379 days

It is probable therefore that at least two complete generations may develop in a year under the most favourable conditions of temperature and

finding of the necessary host, while under unfavourable conditions one generation may require considerably over one year for development.

Summary.

1. *Ixodes holocyclus* is a three-host tick.

2. It is found on native mammals, being abundant on *Perameles nasuta* (the bandicoot), while it has also been found on dogs, cats and man, and exceptionally on the large bush opossum and the rat.

3. Eggs hatch in from 49–61 days in warm weather.

4. Larvae harden and become active in about seven days, and engorge on host in 4–6 days.

5. Larval metamorphosis is prolonged by cold, and is most rapid in warm weather, varying from 19–41 days.

6. Nymphs harden and are quiescent for about seven days, and engorge in 4–7 days.

7. Adults emerge from larval skin in 21–71 days.

8. Coupling occurs away from the host and may last 11 days.

9. Adults harden and seek the host in about seven days and engorge in from 6–21 days.

10. Males are only rarely found on hosts, and have never been found attached or showing signs of engorgement.

11. Oviposition begins 11–20 days after the female leaves the host, and may last from 16–34 days; the number of eggs laid being about 1800–2500.

12. All stages are soon killed by desiccation, gorged nymphs and larvae being very soon killed even at moderate temperature.

13. A mass infestation with *Ixodes holocyclus* has not been seen either on native or domesticated animals.

I have to express my indebtedness to Professor J. Douglas Stewart for the facilities he has afforded me for carrying out this work at the Veterinary School and to Mr D. Selkirk for the trouble he has taken in securing bandicoots and opossums for me.

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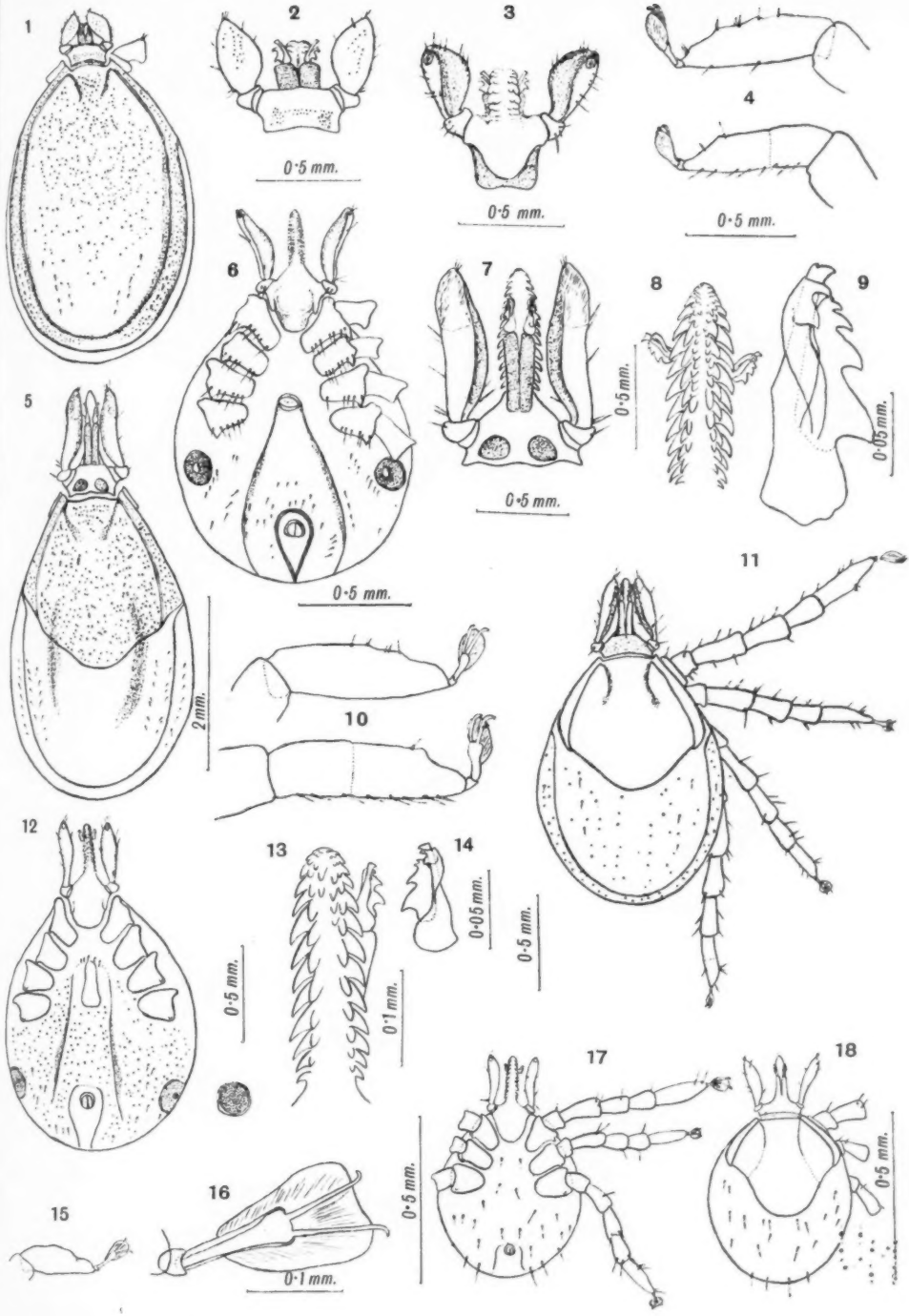
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EXPLANATION OF PLATE XV.

Ixodes holocyclus Neumann, 1899.

Fig. 1. Dorsum of ♂.
Fig. 2. Capitulum of ♂, dorsal aspect.
Fig. 3. Capitulum of ♂, ventral aspect.
Fig. 4. (*a*) tarsus I of ♂; (*b*) tarsus IV of ♂.
Fig. 5. Dorsum of ♀.
Fig. 6. Venter of ♀.
Fig. 7. Capitulum of ♀, dorsal aspect.
Fig. 8. Hypostome of ♀.
Fig. 9. Digit of ♀.
Fig. 10. (*a*) tarsus I of ♀; tarsus IV of ♀.
Fig. 11. Dorsum of nymph.
Fig. 12. Venter of nymph.
Fig. 13. Hypostome of nymph.
Fig. 14. Digit of nymph.
Fig. 15. Tarsus I of nymph.
Fig. 16. Claws and pulvillus of tarsus I of nymph.
Fig. 17. Venter of larva.
Fig. 18. Dorsum of larva.

TESTS WITH *CHARA FOETIDA* AND *C. HISPIDA* ON THE DEVELOPMENT OF MOSQUITO LARVAE.

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The reputed toxic and destructive action of certain species of *Chara* led to the preparation of watery and alcoholic extracts of *Chara foetida* and *C. hispida* which were sent to me with the request that the larvicidal power of these extracts should be tested.

The first supply of extract received arrived during the early winter months when mosquito larvae are rather difficult to obtain. It was therefore set aside until larvae were available.

In December 1923 a large number of *Anopheles bifurcatus* larvae were collected, and with these it was decided to carry out preliminary tests of the powers of the extract. On examination it was found that the extract, after an interval of six weeks, had developed a culture of *Bacillus aceti*, and had a strongly acid reaction. Consequently it was realised that the toxic principles might have been altered and that the results of these tests might not give a true indication of the extract's value.

Nevertheless batches of larvae were placed in measured quantities of stream water and a proportion of the extract was added to each quantity so as to produce the following concentrations: 1 : 2000, 1 : 1500, 1 : 1000, 1 : 750, 1 : 500, and 1 : 250. At the 1 : 250 concentration, soon after the extract was added, the larvae exhibited a spasmodic muscular twitching, and 11 out of the 20 larvae in this batch had died within two hours of the addition of the extract. The remaining larvae, which seemed ill-at-ease, were all alive five hours later, but all had died when they were examined the following morning.

The batch of larvae contained in the 1 : 500 concentration did not develop normally, metamorphosis was delayed, and only five out of the 20 larvae in the batch emerged as rather under-sized adults. The batches in the lower concentrations exhibited no abnormalities either in the rate of development, emergence, or size.

The result of these experiments was therefore entirely inconclusive.

Arrangements were then made to obtain a supply of the living plants. The first specimens were obtained through the Wellcome Bureau of Scientific Research from the Cambridgeshire fens, and the plants were placed in a shallow earthenware trough containing tap water and a few handfuls of earth from

the Horticultural Gardens at Wisley. In this the plants were found to grow exceedingly well, and within a few weeks the trough was filled with a mass of *Chara foetida* and *C. hispida*.

Before conducting experiments with the plants it was thought advisable to remove the tap water from the trough and replace this with the water of the stream from which the larvae were to be collected. The tap water was syphoned off the plants and replaced with stream water having a normal *p*H of 8. The plants were then allowed to continue their growth for a week in the stream water which they did with undiminished vigour, and the *p*H reading was found to remain unaltered. The mean temperature of the water was 68° F.

Fifty *Anopheles bifurcatus* larvae at different instars were now added to the water and kept under careful observation. None showed the slightest sign of distress, and many would lie for hours in actual contact with the surface branches of the plants as they fed at the surface-film. Larvae in stream water associated with the local stream plants were used as a control to this experiment. Both groups developed normally, and if anything, the larvae in the presence of the *Chara* gave rise to larger adults—possibly due to a better food supply, for it was noticed that a copious bacterial and calcium carbonate scum formed constantly on the surface of the water in which the *Chara* grew. This scum had to be repeatedly broken up to enable the youngest larvae to get air at the surface.

After these observations had been completed it was suggested to me by Dr Andrew Balfour that possibly the alleged toxic powers of *Chara* were only developed at higher atmospheric temperatures. Therefore, by means of electric carbon lamps, the temperature of the water and surrounding air was raised to from 75°–80° F., and the experiments were repeated. Except for the fact that the plants during some days grew more rapidly, and the development of the larvae was hastened, the result was similar, and no toxic action on the larvae could be observed.

Later I received from Dr T. A. Henry, of the Wellcome Chemical Research Laboratories, a fresh supply of *Chara* extract. This had been prepared from part of the *Chara* obtained from the Cambridgeshire fens, 1 c.c. of the extract representing 40 grammes of the air-dried plant. The extract on this occasion had been carefully sterilized and neutralized before it was sent to me.

Dilutions of from 1 : 250 to 1 : 1000 were made up with the extract in fresh stream water, and batches of 20 larvae (*Anopheles bifurcatus*) were placed in each dilution. No toxic action was noticeable even in the 1 : 250 dilution, and the larvae developed and emerged in a normal manner. Subsequently the extract was tested on the larvae of *Culex pipiens*, the dilution in this case being with water from a horse-trough in which the larvae were collected. Again no toxic action was noticeable.

Finally, about 100 *Culex pipiens* larvae were added to water containing living plants. In this environment the larvae did not do at all well, but some

pupated and emerged as normal-looking though undersized adults. However, the larvae of *Culex pipiens*, it must be remembered, are not usually found in water inhabited by aquatic plants, but find their most favourable habitat in rain water containing a large amount of organic matter in the process of disintegration by bacterial decomposition—such places, for instance, as rain barrels, gutters, rain-pools in stable yards, horse-troughs, sewage-farms, etc. I am convinced therefore that the fact that the larvae associated with the *Chara* did not do well is to be explained by the influence of an abnormal environment—incidentally water of an alkaline reaction instead of one with an acid reaction—and not by a toxic action on the parts of the plants.

Reviewing the literature on the subject of *Chara* and its alleged lethal powers on mosquito larvae there is considerable evidence to show that the findings of Caballero (1919, 1920, 1922) are not substantiated altogether by the records of other investigators. Briercliffe in Palestine has reported pools covered with *Chara foetida* in which there were large numbers of both Anopheline and Culicine larvae; and Buxton (1924, p. 298) and Heron (1922), also in Palestine, have recorded the same thing.

"In preparing to direct a campaign against yellow fever in Mexico, Vasconcelos noted the following quotation in a recent book: 'An aquatic plant, *Chara foetida*, appears to prevent the development of mosquito larvae in water, according to recent experiments of Caballero. *Chara foetida* which is quite hardy and grows easily, could be employed advantageously in the antilarval campaign in swamps and lagoons.'

"Obtaining specimens of *Chara gymnophylla* from Yucatan, Vasconcelos (*Amer. Journ. Pub. Health*, July 1923) set about to confirm this report, and invited several physicians on the east coast of Mexico to do the same thing. He placed a portion of *Chara gymnophylla* weighing about two kilogrammes in a wooden trough of ten litres capacity in which there was a little earth. An enormous number of Culex larvae developed in the trough within a few days, and a film of coal oil was laid on the water to destroy them. The coal oil and water were then removed by a continuous flow of fresh water into the trough of several hours duration. There was then a new crop of larvae within forty-eight hours, but not one of them was normal. Some were dead, some almost motionless; some floated on the surface of the water and made no attempt to escape. The experiment was continued for two weeks with identical results, in spite of the fact that the water in the trough was 'continuously' changed. It was noticed also that the adult mosquitos resting on the surface of the water in the trough could be easily captured. Cool weather prevented the completion of the experiment. Vasconcelos was sufficiently convinced however to agree with Caballero and Connor that the alga in question secretes a substance that kills mosquito larvae. There are five species of *Chara*, it appears, that have a larvicidal action; *Chara foetida*, *C. contraria*, *C. hispida*, *C. fragilis* and *C. gymnophylla*."

Anyone who has worked with coal oil (paraffin) will know how difficult

it is to remove from vessels, particularly wooden vessels. It therefore seems to me to be most likely that all the coal oil was not actually removed by the continuous flow of fresh water. Paraffin in water has a way of tenaciously adhering to the sides of the container and is practically impossible to dislodge by a stream of water passing over it.

My own conclusions are that the flow of water in Vasconcelos' experiments did not remove all the coal oil, and that the presence of a small quantity poisoned the larvae. I have myself often noticed the sluggishness of adult mosquitoes which have alighted upon pools where a faint trace of paraffin persists. Apart from the direct effect of paraffin upon mosquito larvae and adults in causing death by the occlusion of the tracheae, the vapour has also an indirect poisonous action on larvae and adults.

Hacker (1922) mentions that with experiments on two species of *Chara* in the Federated Malay States he has shown that the plants have no adverse action on mosquito larvae. A third species of *Chara* he thought possibly had some deterrent effect upon mosquito breeding, and therefore he planned to conduct further experiments with this species.

Allaud (1923) states that he found *Chara* spp. a very valuable accessory measure in controlling the development of mosquito larvae in Morocco. *Chara conniviens* was the species studied, and he makes the statement that the virulence of the water in which Chara has grown dies out when the living plants are no longer present.

Maynar (1923) in Spain says he confirms Caballero's results, and that he has found *Chara* to have potent larvicidal properties.

A very interesting paper by Barber (1924) has just reached me giving the results of experiments made by him with *Chara robbinsii* on Anopheline larvae and the larvae of *Aëdes aegypti* (*Aëdes argenteus*). Barber found that *Chara* had no adverse effect on the larvae, and his and my own observations agree very completely.

He summarizes his opinion by saying: "It is evident that whatever larvicidal power may be possessed by some species of *Chara*, this property is not common to the whole genus. It is recommended that in any test of the possible effect of *Chara*, or any other plant, the food requirements of the larvae should be taken into account."

To this I should like to add, that such tests must also be undertaken with a full realisation of how highly important it is that the reaction of the water shall agree with the normal reaction of the water which forms the usual habitat of the particular species of larvae used in such experiments.

Taking the evidence as a whole it is difficult to conclude whether *Chara* is entirely devoid of the larvicidal action imputed to it, or whether under certain conditions, and in certain localities, some species have the power of inhibiting mosquito development, but I am convinced personally by my own experiments and observations that even some of the reputedly toxic species are often devoid of any toxicity whatever.

The whole truth of the subject is obscured by the fact that the experiments of those who have reported a toxic action have not been carried out in such a way as to exclude serious errors. Because in some instances a natural water in which *Chara* is growing can be shown not to harbour the larvae of mosquitoes it is no evidence that *Chara* is responsible for this state of affairs. Moreover, if *Chara* is brought to a laboratory and there allowed to grow, and then mosquito larvae which are added to the water are found either to die or to be adversely affected this again is not conclusive evidence that the plants have any influence upon the larvae. Whether the water has an acid, neutral, or alkaline reaction has been shown to exert a profound influence upon larval development. Certain species do well in water with an acid reaction; others do well only in water with an alkaline reaction, while a third group of larvae of certain species will tolerate, within definite limits, water whose reaction is either acid or alkaline.

Consequently, if these facts are not taken into consideration, and the larvae used in such experiments are not chosen with a view to using only those whose normal habitat, in terms of the *p*H exponent, corresponds with that of the water in which the *Chara* is growing, completely erroneous inferences are likely to be drawn.

As it is, I feel certain that *Chara* can have no generally useful application in practical anti-mosquito measures.

Since the foregoing was written experiments have been conducted to ascertain whether mosquitoes showed any aversion to laying their eggs on the surface of water in which *Chara foetida* and *C. hispida* were growing.

Anopheles bifurcatus, *A. maculipennis*, *Culex pipiens* and *Ochlerotatus rumorosus* were used for this investigation. Batches of the different mosquito species were collected from local stables, and in the woods surrounding the laboratory, and were placed in large insect cages. Each cage held two bowls of water, one containing vigorously growing *Chara* and the other tap-water and grass stalks. With all the species eggs were laid apparently indiscriminately on the water in both bowls, and, moreover, the eggs in each of the bowls hatched quite normally.

Finally experiments were made in the field under natural conditions by planting the species of *Chara* in small natural ponds, and adding mosquito ova and larvae to the water. Not the slightest adverse effect upon the development of the mosquitoes was noticed, and wild mosquitoes continued to oviposit in these ponds.

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SPECIAL APPARATUS AND TECHNIQUE FOR THE STUDY OF MOSQUITOES AND OTHER AQUATIC INSECTS.

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(With 7 Text-figures.)

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In laboratories which are situated either abroad or in districts at some distance from commercial centres, it often happens that needed apparatus is difficult to obtain, and these notes may be helpful to workers in such situations.

Pipettes. Certainly the easiest method of removing larvae and pupae from one container to another is by the use of small pipettes similar to the well-known glass fountain-pen filler. The special features of this apparatus which are desirable are: (1) that the rubber nipple should be thoroughly elastic and have stout walls, so that when the rubber is released after compression with the fingers it will recover its shape almost instantly, thus causing a sufficiently rapid suction to prevent the insect from escaping—as it generally does unless the pipette acts rapidly; (2) that the rubber nipple should not be of large capacity. The ready-made nipples obtainable for pipettes, when new, are usually satisfactory as regards the first essential, but their capacity is often unnecessarily and disadvantageously large. Moreover in the tropics they very soon perish, the inner walls developing an annoying tendency to stick together.

Thick-walled rubber tubing on the other hand can withstand even a tropical climate for much longer periods, and on release after compression will regain

its shape instantly, while the capacity of about one linear inch of the bore is just the right amount to capture a mosquito larva without an undue quantity of water being collected in addition.

To make these pipettes, draw a piece of $\frac{1}{4}''$ internal diameter tubing into pipette-form with a stem about 3″ in length. From glass tubing of the same diameter, cut off a piece about $\frac{1}{2}''$ in length and seal both ends by revolving them in a gas or paraffin blow-lamp flame. Cut off $2\frac{1}{2}''$ of thick-walled rubber tubing of a diameter large enough to slide over and fit the stem of the pipette tightly. Wet the inner walls of the rubber tubing and slip it over the uncontracted end of the pipette so that it envelopes the stem to the extent of one inch. Then wet the small piece of glass tubing which has had its ends sealed, and with this plug the open end of the rubber tube, pushing the glass plug well into the tube. This leaves about one inch of the rubber tube containing an air space which can be compressed between finger and thumb. See Fig. 1.

Fig. 1. To show the construction of the special pipettes. *A*, glass stem; *B*, thick-walled rubber tubing; *C*, glass stopper.

These pipettes have been found to be so preferable to the usual type that I have discarded the use of the latter.

Pipettes for the collection of tree-hole breeding mosquitoes. For the knowledge of this type of pipette I am indebted to Mr F. W. Edwards by whom I first saw it used. I have modified it in one respect, namely the position of the opening, since it was found that if the opening is on the side of the glass wall instead of being at the end of the constricted part there is less likelihood of it becoming blocked by detritus within the tree-hole, and moreover the larvae seem to be more easily sucked into the tube with the aperture in this position.

Obtain a piece of glass tubing about $\frac{1}{2}''$ in internal diameter and 12″ long. Near one end draw off a short stout-walled constriction. This can be done by evenly heating only a small area of the glass tube in the flame, and pulling the ends apart *slowly* when the glass is quite soft. Cut the glass across the constriction, and discard the smaller portion. Heat the tip of the contracted end of the pipette until the opening is closed by the fusion of the glass, and continue heating the tip until a small solid "button" of glass is formed. (This "button" and solid glass end will largely protect the end of the pipette from damage should it strike a hard substance when being inserted into the tree-hole.) Allow the glass to cool, and, on one side only, heat a small area of the glass about $\frac{1}{2}''$ from the solid tip. When the glass softens, keep it steadily in the flame and blow with considerable force down the open end of the tube so as to explode the glass in the softened area. This will result in a small hole being formed in the glass wall. By playing the flame over the hole any projecting

glass can be made to contract to a uniform level, and the edges of the hole will be thickened. A hole from $\frac{1}{8}''$ to $\frac{3}{16}''$ in diameter is required. To the other end of the pipette a large thick-walled rubber bulb is fitted—such as are used for aural syringes. If an aural syringe bulb is unobtainable the bulb of a motor horn makes a good substitute when the open end of the bulb is made to fit the diameter of the tube by a suitable plug. These pipettes will be found invaluable in the collection of mosquito larvae, etc., from small holes in trees and rocks. Fig. 2.

Fig. 2. Pipette for collecting tree-hole larvae and pupae.

Glass rearing-dishes. In rearing larvae it should not be overlooked that the health of the living larvae and pupae is menaced by the presence of dead and dying specimens. Larvae are subject to infectious diseases of many sorts, and one victim can spread these diseases to others. It is therefore of the utmost importance when larvae are being carefully reared to remove dead or diseased individuals immediately they are observed, and if the mortality increases, the remaining larvae should be transferred to fresh water which has previously stood in sunlight for several hours. These larvae should be collected by the pipette and be stranded on clean blotting paper until most of the water has been absorbed, when they may be removed from the paper and placed in the fresh water by means of a section lifter. (Incidentally, I have at times successfully checked the spread of disease by placing the living and healthy looking larvae in 70 per cent. alcohol for thirty seconds before their transference to sun-sterilized water.)

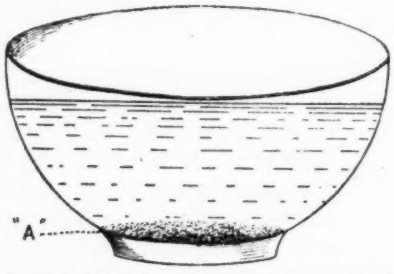


Fig. 3. The finger-bowl rearing-dish. *A*, detritus at the bottom of the centre of the bowl.

Glass jam jars, although commonly used, are not satisfactory things in which to rear mosquito larvae: those that have mouths of reduced diameter make the capture of the larvae by the pipette difficult, and dead larvae, for the same reason, cannot easily be removed. Glass beakers, while they make very good rearing-dishes for some species, are usually made of too thin a glass to prove generally satisfactory.

An ideal rearing-dish for larvae of most species is to be found in the plain (and cheap) glass finger-bowls which are usually obtainable everywhere, particularly in the tropics where fruit happily constitutes so large a portion of man's diet. Finger-bowls present a relatively extensive water surface, while the depth of the water, even when the bowl is full, is not excessive, and the larvae and pupae, if necessary, are thus easily kept under observation. Moreover the semi-spherical curvature of the bowl causes detritus, and any dead larvae or pupae which sink, to be concentrated at the centre of the bottom of the bowl—a position from which unwanted material is easily removed. Fig. 3 will make this plain.

Special creosote bottles for storage boxes. Naphthalene which is so often used in entomological storage boxes to preserve the contained specimens from the

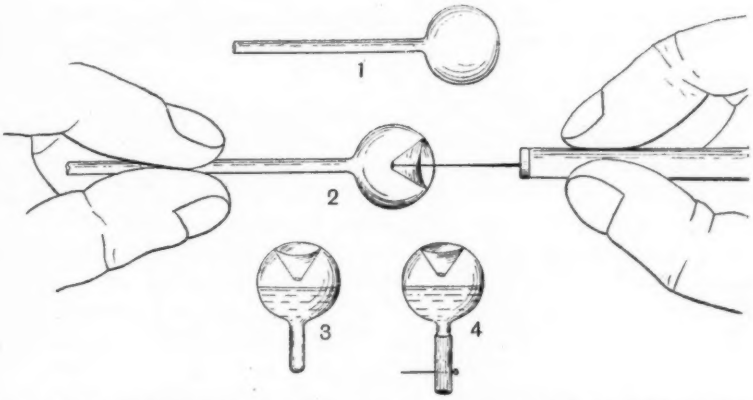


Fig. 4. Method of the construction of the special creosote bottles. 1, glass tubing with bulb blown on one end. 2, depressing the softened upper surface of the bulb. 3, the bulb cut off from the tube, sealed, and filled with creosote. 4, rubber tubing and pin attached ready for insertion in the specimen box.

destructive attentions of other insects fulfils this function fairly well in temperate climates, but in hot weather, and particularly in the tropics, naphthalene volatilizes rapidly and has to be frequently renewed. In addition naphthalene has little or no control over the growth on the specimens of fungi which in the tropics are more destructive to collections than even the small insect pests. Creosote, in place of naphthalene, safeguards the collections both from fungi and insect pests. As creosote is a liquid, however, its application to the storage boxes has involved difficulties. Special safety bottles are made which may be placed in the storage boxes, but they are often difficult to obtain, and most types are somewhat cumbersome. Pieces of cotton wool are often pinned into the boxes, and a few drops of creosote poured on to the wool, but this is a most unsatisfactory method as the creosote is apt to spread on the inner surface of the box and cause a very untidy appearance.

I have found that neat and thoroughly serviceable creosote bottles can be made without difficulty from glass tubing. If pinned into one corner of the boxes these bottles take up very little room, and do not spill their contents, no matter in what position the box is placed. The bottles are made in the following way. Fig. 4.

Obtain a piece of glass tubing about $\frac{1}{8}''$ in internal diameter. Seal one end by revolving it in a gas or paraffin blow-lamp flame, and when the lumen has been completely closed heat to a bright red colour, and, by blowing carefully down the open end of the tube, form a spherical bulb about $\frac{1}{2}''$ in diameter. Allow the glass to cool and transfer the glass tube with the bulb at one end to the left hand. In the right hand take a mounted dissecting needle and bring the upper surface of the bulb carefully into the flame once more until the glass softens. Then remove the bulb from the flame, and rapidly, but with a steady pressure, strike the softened glass exactly in the centre with the point of the needle, and press the glass inwards until the point of the needle reaches to the centre of the sphere. This results in the formation of a semi-spherical bulb with an invaginated centre, similar to the familiar safety ink-pot.

Allow the glass to cool and with the needle carefully break through the glass at the middle of the depressed centre, forming a hole about $\frac{1}{16}''$ in diameter. Now cut off the bulb from the glass tubing, leaving about $1''$ of tube attached to the lower end of the bulb, and seal this end of the tube by revolving it in a flame. Over the sealed end of the tube a small piece of rubber tubing is slipped, leaving $\frac{1}{4}''$ of the rubber tubing extending beyond the sealed end of the glass. Half fill the bottle with creosote by means of a capillary pipette whose end will enter the hole in the centre of the bulb, and the bottle, lying on its side, may then be pinned through the rubber into one corner of the box. These bottles have been found to act admirably: the creosote evaporates rapidly enough to preserve the specimens perfectly, although one charge of creosote will last for more than a year.

A method of keeping malaria-infected and other fed mosquitoes in a favourable environment. In the tropics particularly, mosquitoes that are used in malaria transmission experiments are apt to die when they are necessarily imprisoned in small glass tubes owing to the dry state of the atmosphere within these tubes. To add a few drops of water to the tube usually results in the death of the captive mosquitoes either by supersaturation of the contained air or by the whole of the inner walls of the tube becoming wet by condensation. The optimum conditions for the life of a fed mosquito are, that the insect shall rest comfortably, that there shall be a free play of air within the tube, and that while the air within the tube shall contain a high concentration of water vapour it shall yet not contain sufficient to reach the dew-point of the surrounding atmosphere. These optimum conditions are attained by the following method. Fig. 5.

The *unfed* mosquitoes should be placed in glass tubes measuring $4''$ in length by $1\frac{1}{4}''$ in diameter—a common size of specimen tube. Before the insects are

placed in the tube a small dried twig should be inserted in such a way that the upper end of the twig does not reach quite to the top of the tube. Place the unfed insects—not more than three together—in these tubes and close the mouths of the tubes by a small square of mosquito-netting tied over the open end with cotton or light string. Do not use rubber bands to hold the mosquito-netting in place, as the rubber rapidly perishes, and by breaking, may unexpectedly release the insects. Allow the mosquitoes to feed by inverting the tube over the patient's skin, and when they have fed arrange the tubes in a line on a table. Across the tops of the tubes stretch a length of ordinary flat lamp-wick, about $\frac{3}{4}''$ in width, so that the mouths of the tubes are not entirely covered by the wick. One free end of the wick is now to be placed in a bowl containing water which by capillarity will be drawn along the wick over the tubes and so keep the air within near the point of saturation. A small stone should be placed on the wick to hold it in position over each tube, and by raising or lowering the height of the bowl water-reservoir the flow of the water can be exactly con-

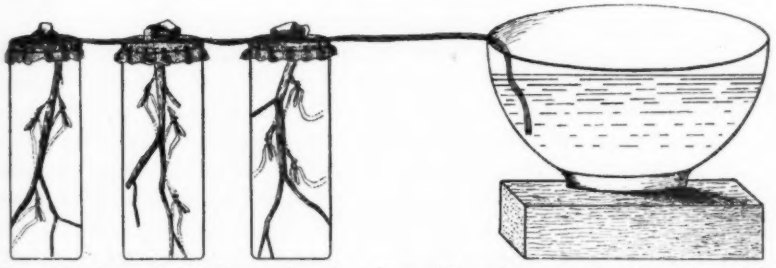


Fig. 5. Keeping malaria-fed mosquitoes in a favourable environment. The disposition of the apparatus.

trolled. If the tubes are spaced in line an inch apart each length of wick will be found to serve up to a dozen tubes satisfactorily. By this means I have found it possible to keep malaria-fed mosquitoes alive even in exceedingly hot dry atmospheres when it has been found impossible to do so in other ways. Half a raisin should be placed on one side of the wick on the uncovered portion of the mosquito netting: the raisin is kept constantly moist and provides the insect with fruit sap which I have found advantageous both to the life of the insect and apparently also to the development of the plasmodia.

A method of treating the stomachs of malaria-fed mosquitoes. After dissection by one or other of the approved methods the stomach will be seen to be either empty and flat or, if the insect has had blood meals since the first infected meal, it may be partially distended with blood. In either case young oöcysts may be overlooked unless precautions are undertaken. In empty stomachs which in the process of being withdrawn from the body have been extended, much of the contained gas or air is forced out by the contraction of the diameter. When the removed stomach is freed from tension it attempts to resume its normal shape, but the weak contraction of the muscles and the partial vacuum

within often leaves it completely flattened. If the oöcysts are numerous or well developed they can still be seen quite easily, but if they are young and few in number it may well happen that what few there are lie on the upper or lower flattened surfaces of the organ, and they are then very easily overlooked. The flattened stomach may be made to resume its normal shape in the following way.

On a clean microscopical slide place a drop of water large enough to accommodate and submerge the stomach. Transfer it to the water on the slide, and with a rocking motion to prevent the organ adhering to the glass, gently heat the under surface of the slide. As the water warms up the stomach muscles undergo a vigorous contraction which causes water to be drawn in, and it resumes its normal pyriform shape. The careful application of heat to the under surface of the slide should cease immediately the contraction of the stomach is observed. If the stomach is then examined under the microscope as it lies in the water, any oöcysts projecting from the curvature of the outer wall are conspicuous, and by rotating the stomach with the aid of a needle, every part of the wall may be examined.

If the stomach contains blood make a small incision in the wall and place it in a large drop of water on a clean slide. To this add a drop of 70 per cent. alcohol. Powerful diffusion currents will be set up which effectually evacuate the stomach of blood through the aperture made in the wall. If necessary the process may be repeated by placing the organ in another drop of water and alcohol before it is finally placed, when free from contained blood, in a drop of clean water on a slide and heated carefully in an attempt to get it to resume its normal shape—as was done in the case of the empty and flattened stomach. Nine times out of ten it will be found that contraction of the muscle walls takes place. The stomachs may be satisfactorily preserved either by mounting them in cell slides in the formaldehyde-borax solution which is mentioned elsewhere in this paper or by placing them in small glass tubes—such as Wassermann test-tubes—containing the solution.

A method of demonstrating the mosquito wing-venation. In some species of mosquitoes the complete wing-venation is difficult to see even with the aid of a binocular dissecting microscope. This is especially true of the cross-veins and supernumerary veins. The whole system is easily made conspicuous however by removing the wings from the body of the insect and soaking them overnight in warm undiluted carbo-fuchsin solution for about 12 hours, and subsequently washing the wings rapidly in 70 per cent. alcohol. The wing membrane will be found to lose all the stain in the alcohol while the veins retain it, and are stained a brilliant fuchsin red.

The use of chloroform vapour for killing mosquitoes preparatory to mounting. Unquestionably the most satisfactory method of mounting mosquitoes is upon the celluloid stage: a method that is now generally adopted. Apart from the rigidity and the neat appearance it affords, the transparency of the stage enables even the under surface of the specimen to be examined. For mounting

mosquitoes a useful size for the celluloid stage is 9 × 12 mms. The fine pin on which the insect is impaled should be a No. 19 (white), and the stage supporting pin should be No. 16 (white)—D. F. Taylor and Co., London. The use of artist's gelatin in place of celluloid has been advocated owing to the latter material's inflammability, but the risk of accident with celluloid in a laboratory which normally contains many equally inflammable substances is negligible, and although the use of artist's gelatin in temperate climates is satisfactory, in the tropics it is difficult to obtain and has moreover a tendency to soften and become opaque in some climates. On the other hand celluloid remains transparent and hard even in hot steamy climates, and the now ubiquitous motor garage can usually supply sheet celluloid so commonly used for motor-car windows, and for the repair of celluloid accumulator cases.

In killing mosquitoes preparatory to mounting them on these stages chloroform vapour should be used. Chloroform is highly toxic to insects and its effect takes place in two phases, both of which can be made use of. The mosquitoes which are to be killed should first be placed in a wide mouthed bottle or large specimen tube closed with mosquito-netting. The tube should be placed upright on a table, and the open bottle of chloroform should be held over the netting at such an angle that only the heavy chloroform vapour falls into the tube. Almost immediately the insects are stunned, and just as soon as this occurs and all the insects are paralysed, they should be carefully tipped out on to a clean piece of smooth surfaced paper. This, the first phase of the action of the chloroform, has a remarakble action on the wing muscles of the insects, in that the wings, in the majority of instances, are extended in a fixed position at right angles to the body. In those insects which do not exhibit this position of the wings, it will be found that they are easily placed in fixed extension by means of a pin or dissecting needle. By laying each insect on its back and inserting the point of the fine needle through the ventral surface of the thorax between the coxae, the insects should be mounted as rapidly as possible, and be then transferred to concentrated chloroform vapour, for from three to five minutes. The concentrated chloroform vapour is obtained by saturating a piece of cotton wool in chloroform, and placing it under a small bell-jar. The effect of the strong vapour is to kill the insects rapidly and to bring about a lasting muscular rigidity which holds the wings extended until desiccation of the body takes place. It is important not to leave the insects in the concentrated chloroform vapour for longer than from three to five minutes, otherwise the celluloid is apt to become coated with a crystalline deposit of camphor derived from the constituent camphor of the celluloid.

Floating egg-harbours. If the eggs of mosquitoes are allowed to float on the surface of water while they are undergoing development they naturally tend to drift to the sides of the container, and by evaporation of the water with the consequent lowering of its level, are apt to become stranded and killed by desiccation. This can be avoided by cutting out the centre of a large cork with a $\frac{1}{2}''$ diameter cork-borer, and subsequently cutting the cork across its

width into cork rings about $\frac{1}{8}$″ thick. These rings should be soaked in molten paraffin wax to render them waterproof, and then be placed on the water. The eggs are easily lifted from the water surface with the aid of a wet camel's hair brush, the tip of the brush being brought up under each egg, and in this manner they are transferred to the water surface within the cork ring. Obviously, any fall or rise in the level of the water will not now affect the eggs as the cork harbour will always rest on the surface, while the larvae which hatch, by naturally descending, escape from the confines of the ring into the main body of the water. Fig. 6.

Preserving larvae and pupae. Each worker usually has his own favourite method, and there are many excellent methods. Larvae and pupae that are to be preserved should never however be placed while alive into alcohol, and if avoidable alcohol should not be used as a preservative. It causes hardening and distortion of the body, and other objectionable changes. If there is no alternative to its use the insects should be killed by placing them first in hot, *not boiling*, water from which they may then be transferred to one part of the alcohol diluted with one part of water, and six hours later be placed in the

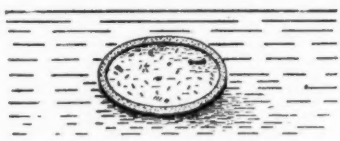


Fig. 6. A floating egg-harbour. Cork ring enclosing the floating ova.

undiluted alcohol. The use of boiling, or nearly boiling water to kill larvae and pupae, although so often used, is a great mistake: the high temperature causes the air contained in the tracheae and other parts of the body to expand so rapidly that the shape of the insect is often badly distorted, and the body wall is consequently often ruptured. The water used for killing larvae and pupae should only reach a temperature that feels just distinctly hot when a finger is dipped into it.

Larvae and pupae which have been killed with hot water (undoubtedly the best way of killing them preparatory to preservation) will be found to keep perfectly preserved for years in the following solution:

4 per cent. Formaldehyde solution = 10 cc. of 40 per cent. Formaline.
0·5 per cent. Borax solution = 10 cc. of a 5 per cent. solution of Borax in distilled water.

Make up to 100 cc. with distilled water, and add 2 cc. of glycerine.

Rearing individual larvae or pupae. In localities where new species may be discovered it is of the greatest importance to rear single specimens of what is thought to be a new larva or pupa, under conditions made as favourable as possible to the development of the insect. If a rare larva or pupa is placed in

one of the ordinary rearing-dishes there is considerable risk of its being lost sight of in the presence of other larvae, while the rare specimen is also largely left exposed to the ordinary risks to its life.

Rare larvae or pupae, or any particular specimen which is required to be raised to the adult state, under conditions that as far as possible ensure its development, should be placed separately in glass tubes containing a supply of the water in which the specimens were found. The water before it is placed in the tube should be poured into a clean white porcelain photographic dish. Any dead mosquito larvae, or other living insect larvae, aquatic mites, or minute crustacea, if present, should then be removed from the water. About one third of the capacity of the specimen tube may now be filled with this

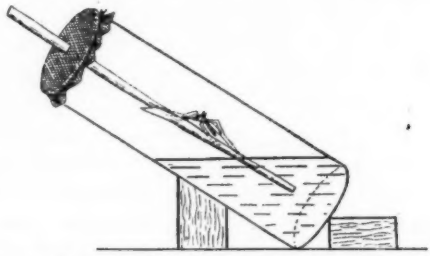


Fig. 7. Apparatus for rearing individual larvae or pupae.

water, and after the specimen to be reared has been added, the open mouth of the tube is closed by tying over it a small square of mosquito-netting. Incline the tube at an angle of 45° supported on two wooden or cork blocks—as indicated in Fig. 7— and place it under lighting conditions comparable with those of the natural breeding-place. In the case of tree-hole larvae and pupae the tube should be shaded with a screen of opaque paper.

As soon as the specimen reaches the pupal state insert through the mosquito-netting a piece of grass or straw to afford the adult insect a support on which it may climb from the water-surface when it emerges from the puparium. Insects allowed to develop this way usually emerge as very perfect specimens, and as they have little inclination to fly about the confined space within the tube, the ornamentation thus remains undamaged. In the case of predaceous species they will have to be fed, of course, regularly with other mosquito larvae until pupation occurs.

NEW FILARIAE FROM INDIAN BIRDS.

By ASA C. CHANDLER, Ph.D.

(*From the Hookworm Research Laboratory (supported by Indian Jute Mills Association), Calcutta School of Tropical Medicine.*)

(With 10 Text-figures.)

In the body-cavity of a Racket-tailed Drongo (*Dissemurus paradiseus*) which died at the Calcutta Zoological Gardens, and the blood of which was teeming with two species of microfilariae, were found males of two new species of filariae, together with a single female specimen. In a Hunting Cissa (*Cissa chinensis*), which died at about the same time, males of one of the species discovered in the Drongo were found again, accompanied by a single female different from that found before. Since this cissa also harboured two species of microfilariae in its blood, the identity of either of the females is doubtful. In another specimen of hunting cissa a single male specimen of an entirely different species, which I have made the type of a new genus, was found. Here again two species of microfilariae were discovered so that it is not possible to say which is the offspring of the adult found. It had been hoped that more infected specimens of these birds might be obtained so that the confusion as regards relationships of the females and offspring to the males might be cleared up, but there appears to be little hope of more being obtained in the near future.

Hamulofilaria new genus.

Male of small size, white in colour. Cuticle smooth except for striated areas near cloaca. Tail bent like a grappling hook and sharply pointed, without alae. Near tip of tail a roughened callosity. Spicules unlike in size and shape, the larger one resembling a curved surgical needle, the shorter one trough-like. Head blunt, the oesophagus preceded by an oral cavity surrounded by a thickened wall presenting the appearance of lips when seen in optical section. Four papillae surround the mouth. *Female* unknown.

Hamulofilaria indica new species.

Male, 7·5 mm. in length, with a maximum diameter of 215μ. Cuticle smooth except for a distance of about 200μ just anterior to the cloaca on the ventral side, and for a somewhat shorter distance a little farther forward on the dorsal side, where there are conspicuous transverse striations which do not completely encircle the body. Head with a distinct oral cavity 22·5μ

deep and about 15 μ in diameter, surrounded by a thickened wall resembling lips when seen in optical section. Four papillae surround the mouth. The oesophagus is 875 μ in length with a maximum width, near its posterior end, of 144 μ. The nerve ring is 180 μ and the excretory pore 310 μ from the anterior end. The tail is not coiled but is bent in the form of a grappling hook, tapers to a sharp point, and is 200 μ in length with a diameter, at the cloaca, of 72 μ. Near the tip of the tail on the ventral side is a roughened callosity about 40 μ in length. The longer spicule is slender and is shaped like a curved surgical needle; it is a closed tube up to a point not far from the tip. It is 310 μ in length with a maximum width of about 16 μ. The shorter spicule, which is trough-shaped and serves as a guide for the longer one, is 130 μ in length; it has a pair of conical guards near its tip. The roughened callosity and the striated areas are apparently for the purpose of holding the body in position during copulation. The vas deferens is markedly constricted at its junction with the ejaculatory duct about 68 μ anterior to the cloaca. *Female* unknown.

Only a single male specimen found, lying on the mesentery in the vicinity of the liver in the abdominal cavity of a hunting cissa, *Cissa chinensis*, which died in the Calcutta Zoological Gardens. Blood-films from this bird contain two species of microfilariae which are described below as *Mf. cissae* and *Mf. comma*. It is not possible to say which is the offspring of *Hamulofilaria indica*.

Filaria bosei new species.

Male 9 to 11 mm. in length, with a maximum diameter of about 150 μ; the head is 80 μ in diameter. Cuticle smooth and body white. Mouth simple, without papillae. Oesophagus stout, about 650 μ long, *i.e.* about 1/15 of body length. Nerve ring 130 to 140 μ from anterior end. Tail 140 to 160 μ in length, about 1/65 of body length, moderately coiled, tapering and bluntly rounded at end, without alae. There are three pairs of inconspicuous postanal papillae situated about 40, 75 and 130 μ respectively behind the cloaca. The spicules are approximately alike, short and stout, in the form of curved troughs; they are from 70 to 80 μ in length and about 21 μ in maximum breadth.

Female (*F. bosei*?), 28 mm. long, stout bodied, with a maximum diameter of 730 μ, maintained for the greater part of the length. Cuticle smooth. Mouth simple, without papillae; the head 160 μ in diameter. Oesophagus stout, 980 μ long (about 1/28 of body length), gradually increasing in diameter to its posterior end. Nerve ring 200 μ and excretory pore 320 μ from anterior end. Body widens distinctly at vulva to a diameter of 300 μ; the vulva is situated 450 μ from the anterior end. The tail is short and bluntly rounded, the anus being about 200 μ from the tip. The vagina consists of a gradually widening pouch of large size, reaching a length of 5 mm. and a maximum width of 500 μ. Behind the pouch is a very marked constriction which divides into the two uteri; these very gradually enlarge and do not reach their full diameter before a point a little behind the middle of the body. One uterus turns forward at about three-fifths the length of the body from the anterior

end, runs forward as a very wide tube to a point about the middle of the length of the vagina, where it constricts to a narrow ovarian tube. The latter runs forward to a point just anterior to the vulva, turns backward again, and terminates just behind the region where it first turned forward, about 17 mm. from the anterior end. The other uterus continues backward as a broad tube to a point about 2·5 mm. from the posterior end, abruptly narrows on the curve to an ovarian tube, runs forward to a point about 12·5 mm. from the anterior end, turns back again and ends at the same level as the other oviduct.

Only one female was found, this one in a specimen of *Dissemurus paradiseus* in which a single male of *F. bosei* and two males of *F. brevicauda* were found. It lay on the peritoneal covering of the axillary air sac. Since, as explained above, a different female was found in a cissa in which *F. brevicauda* but not *F. bosei* occurred, it is probable that the female above described belongs to the species *F. bosei*, although the occurrence of two kinds of microfilariae in the cissa throws some doubt on this. I have named this species in honour of Rai Bahadur B. Bose, of the Calcutta Zoological Gardens, through whose co-operation the specimens were obtained.

Filaria brevicauda new species.

Male 7·5 to 10·5 mm. in length, with a maximum diameter of from 160 to 180 μ, bluntly rounded at each end. Cuticle smooth. Mouth simple, without papillae; the head 92 to 95 μ in diameter. Oesophagus 400 to 480 μ in length, about 1/20 of body length. Nerve ring 148 to 160 μ, and excretory pore 280 to 290 μ, from anterior end. The tail is very short, curved, conical in form, and bluntly rounded at the tip; it is from 60 to 65 μ in length with a diameter, at the cloaca, of 55 μ. There are two pairs of inconspicuous papillae, one preanal and the other postanal. The short stout spicules are unequal and are trough-like in form. The longer one is 106 μ in length, the shorter one only 65 μ.

Female (*F. brevicauda*?) 18 mm. in length, stout bodied, with a maximum diameter of about 450 μ. The diameter of the head is the same as the diameter at the anus, 128 μ. Mouth simple, without papillae. Cuticle smooth. Oesophagus slender, about 600 μ in length (1/30 of body length), with a maximum diameter of 32 μ. Nerve ring 192 μ and excretory pore 520 μ from anterior end. The vulva is situated 380 μ from the anterior end, and is not marked by any prominence or enlargement of the body. The intestine at its beginning is equal in width to the oesophagus, but it rapidly widens out, in the form of a cone about 320 μ long, to a width of 128 μ and is filled with globules evidently ingested, but these globules are not, as at first suspected, blood corpuscles. The bluntly rounded tail is 160 μ in length.

A single female specimen was found in a cissa when the liver was being teased out, accompanied by a male *F. brevicauda*. Whether these specimens were in the substance of the liver or on its surface under the mesentery is not certain. Another male of the same species was found in the body cavity, near the kidney, of the same bird. The fact that there were two species of

microfilariae in the blood of this bird leaves some doubt as to whether the female here described is *F. brevicauda*, but this seems highly probable.

In the drongo in which *F. bosei* and *F. brevicauda* were found, two species of microfilariae were present in the blood. These are described below as *Mf. colubroides* and *Mf. cephalocauda*, since it is not possible to assign them to their respective parent forms until birds infected with only one filaria are found. The two types of microfilariae which occurred in the cissa in which only *F. brevicauda* was found were only studied in the living state, the films having subsequently disappeared. One of these microfilariae was noted as being very long with pointed tail and loose sheath, from which it seems probable that it was identical with *Mf. cissae*, and not with either of the embryos found in the drongo. The other species was merely noted as being smaller and sheathed. Two species of microfilariae found in the cissa which contained *Hamulofilaria indica*, which I describe below under the names *Mf. cissae* and *Mf. comma*, are both distinctly different from the forms found in the drongo. There is nothing to indicate which one is the offspring of the adult species found in this bird.

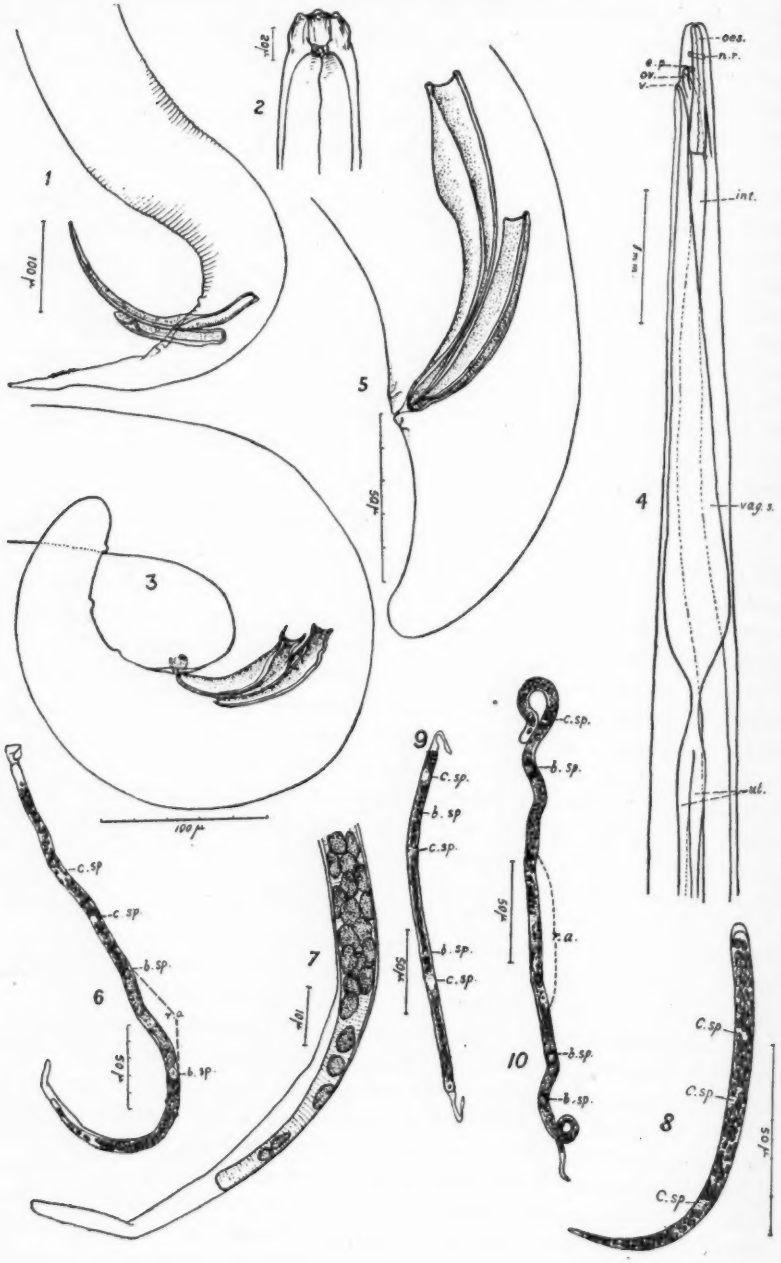
Microfilaria cephalocauda new species.

Length 180 to 205μ, with a diameter of about 4·8μ. Body after death lies in a straight line or bent in a broad curve. Sheath present, extending beyond the body about 10 or 12μ at each end, but showing a tendency to collapse as shown in Fig. 9. Body of almost uniform width throughout, the head and tail both bluntly rounded and quite alike in shape. Nuclei in three irregularly disposed rows. Three clear spots formed by breaks in the column of nuclei at about 1/9, 1/3 and 3/5 the length of the body from what I presume to be the anterior end of the body. A short distance anterior to the second spot, sometimes about midway between the first and second, and also immediately anterior to the third spot, there is an area, evidently a large cell occupying the entire width of the body, which stains deep blue with Romanowsky stain, and has fine red stippling in its centre.

This species occurs in abundance in the blood-films of the drongo, *Dissemurus paradiseus*, in which *F. bosei* and *F. brevicauda* were found.

Microfilaria colubroides new species.

Length variable, from 280 to 350μ, with a diameter of about 6·5μ. No sheath visible. Body after death lies in irregular short angular curves. Head bluntly rounded, tail slender and pointed, giving a strikingly snake-like appearance. Head devoid of nuclei for about 7 or 8μ; usually the most anterior nucleus is more or less isolated. Nuclei very indistinctly demarcated. A break in the nuclei occurs about 50 to 60μ from the anterior end. A region in the middle of the body or a little posterior to the middle, for a distance of about 50 to 65μ, stains deep red (Romanowsky stain) in contrast to the rest of the body. In this red area there are abundant red granular spots as well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as irregularly scattered nuclei. End of tail free from nuclei. Three blue-staining spots, one about 80 μ from anterior end, the other two behind the red area, about 30 μ apart.

This species was found in the blood-films of a drongo (*Dissemurus paradiseus*) along with *Mf. cephalocauda*, but in less abundance.

Microfilaria cissae new species.

Length 300 to 365 μ exclusive of the sheath, which extends from 7 to 14 μ beyond the anterior end of the embryo, and from 35 to 46 μ beyond the posterior end. Diameter about 7 μ. Body after death lies stretched out straight or in broad curves. Head bluntly rounded, tail tapering almost to a point but truncate at the tip. Nuclei irregularly disposed in about three rows. Last 30 or 40 μ of tail end with scattered nuclei in a single row usually 5 or 6 of them, but arrangement irregular, sometimes last one at very tip of tail, sometimes at some distance from tip. Head end free of nuclei for a distance of from 7 to 14 μ. A little posterior to the middle of body there is a region about 50 to 70 μ in length occupied by a red-staining body without distinct nuclei; at each end of this a large round cell-like body which stains blue. Usually a break in the column of nuclei about 1/4 length of body from head end and sometimes another about at the junction of anterior and middle thirds of body. Body finely striated, but the striations distinct only at tail end a short distance from tip.

Common in blood films of hunting cissa, *Cissa chinensis*, in which a single male specimen of *Hamulofilaria indica* was found. *Mf. comma* was also found in the same blood, but was much less abundant.

Microfilaria comma new species.

Length 108 to 114 μ, with a maximum diameter of from 5·5 to 6 μ. A tight fitting sheath present, obvious only at anterior end where it exceeds the

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LEGENDS FOR FIGURES.

Fig. 1. *Hamulofilaria indica.* Caudal region of male, showing roughened callosity near tip, spicules, and striated portions of cuticle.

Fig. 2. *Hamulofilaria indica.* Head, showing oral cavity.

Fig. 3. *Filaria bosei.* Caudal region of male, showing three papillae and spicules.

Fig. 4. *Filaria bosei.* Anterior region of female. Abbrev. as follows: *e.p.* excretory pore; *int.* intestine; *n.r.* nerve ring; *oes.* oesophagus; *ov.* anterior limit of ovarian tube; *v.* vulva; *vag.s.* vaginal sac; *ut.* uterus.

Fig. 5. *Filaria brevicauda.* Caudal region of male, showing papillae and spicules.

Fig. 6. *Microfilaria cissae.*

Fig. 7. *Microfilaria cissae.* Tail, much enlarged.

Fig. 8. *Microfilaria comma.*

Fig. 9. *Microfilaria cephalocauda.*

Fig. 10. *Microfilaria colubriformis.*

Lettering to Figs. 6, 8, 9, 10: *b.sp.* blue-staining spot, with red stippling; *c.sp.* clear spots formed by breaks in the nuclear column; *r.a.* red-staining area.

embryo by a few micra only. Body after death comma-shaped. The head is bluntly rounded and the tail pointed; the body begins to taper at about the middle of its length. Nuclei disposed in three irregular rows; at the head end there are three nuclei slightly separated from the main column of nuclei. There is usually a break in the nuclei about 33 to 35 μ from the anterior end, but it is not always apparent. Another break may occur near the middle of the body, and a third at the junction of the third and last fourths of the body. The body is very faintly striated.

Occurs sparingly in blood-films of *Cissa chinensis*, accompanied by *Mf. cissae*. Male *Hamulofilaria indica*, only adult found.

NOTES ON THE STREBLINAE, A SUBFAMILY OF THE STREBLIDAE (DIPTERA PUPIPARA).

By QUINTA CATTELL KESSEL, M.Sc.

With an Introductory Note by Hugh Scott, Sc.D.

(With 9 Text-figures.)

[Introductory Note. The following paper is part of the result of work done in the University Museum of Zoology, Cambridge, between Nov. 1922 and June 1923. The present writer has for some years been receiving specimens of Nycteribiidae for determination, and with them there had accumulated at Cambridge a small collection of another family of bat-parasites, the Streblidae. At the writer's suggestion, Mrs Kessel undertook a systematic examination of this collection, and with it of material kindly lent by the British Museum through Major Austen and Mr F. W. Edwards, by Professor Poulton from the Hope Museum, Oxford, by Dr G. A. K. Marshall from the Imperial Bureau of Entomology, and by Mr S. Hirst. To all these gentlemen thanks are tendered, also to the authorities of the British Museum for the gift of certain named duplicates, and to Mr Oldfield Thomas for checking the names of the mammalian hosts. Thanks are also due to Mr Gillings for the care exercised by him over the drawings, and to the editors of *Parasitology* for accepting this paper for publication.

Since leaving England Mrs Kessel has continued her study of this family in the Department of Entomology, Cornell University, receiving material from still further sources. She hopes soon to publish in America an up-to-date synopsis of the whole family. The present paper, based principally on part of the material in the British Museum, comprises only a portion of the results of her work, being a detailed study of one subfamily. Numerically this section forms merely a small part of the Streblidae, but its members are structurally very remarkable: this will be seen from the descriptions and figures, which show the extraordinary form of the head, antennae and palpi, the complexity of the chitinous pieces composing the head and the remarkable ctenidium on its underside, as well as other striking features.

The family Streblidae was separated from Hippoboscidae by Kolenati in 1863, and an important revision of it was published by Speiser in 1900 (*Arch. Naturg.* 66. I, pp. 31–70, pl. iii–iv). These insects are almost all parasites on bats (the present paper contains one record from an opossum, *Glironia venusta*, Thos., and *Strebla avium* is recorded from doves and parrots), but

unlike NYCTERIBIIDAE, which are quite wingless, most STREBLIDAE have wings, either fully developed or more or less reduced (see Speiser, *op. cit.* p. 49, figs. 1–8), though in one genus at least (*Paradyschiria*) these organs are entirely absent. In some, at any rate, of the forms with fully developed wings, these organs are, in repose, folded in a rather complex manner (as shown by Speiser), laid in a bristle-free space on the back of the abdomen, and probably held in place by bristles at the sides of the latter, which project over the wings; a method of packing which may protect the wings when the insect is crawling about among the bat's fur.

STREBLIDAE are, like HIPPOBOSCIDAE and NYCTERIBIIDAE, "pupiparous" (*i.e.* giving birth to a full-grown larva which immediately pupates), not oviparous as stated by Kolenati. This was shown by Speiser, and more recently the larva of *Nycteribosca amboinensis* has been described and figured by Muir[1]. Otherwise not much appears to be known of the biology of the family as a whole. As in NYCTERIBIIDAE, a single species of STREBLIDAE may be parasitic on bats of several different species and genera; while a single bat-species, and probably even a single bat-colony or bat-individual, may harbour both NYCTERIBIIDAE and STREBLIDAE. These facts are shown in tabular form by Falcoz[2] and are also indicated in certain unpublished records before me. The phylogeny and geographical distribution of the family have been discussed in various writings of Speiser, etc., but need not be entered into here.

The extraordinary insect *Ascodipteron*, previously regarded as belonging to a separate family, ASCODIPTERIDAE, has more recently been referred to STREBLIDAE. The life-history of one species is related by Muir (*op. cit.*); males and females newly emerged from their puparia have fully-developed legs and wings, but at a later stage the female bores its way into the skin of the bat with the aid of a series of cutting-blades on its proboscis, loses its legs and wings almost entirely (only the stumps being left) and becomes almost completely encysted under the skin of the host, merely the hind end of the abdomen remaining external. The imbedded female produces a full-fed larva, which falls to the ground and pupates. Most species of *Ascodipteron* are known only in the female sex and the encysted phase.—H. S.]

This discussion of three members of the subfamily STREBLINAE, of the family STREBLIDAE, is an attempt to dispense with the confusion between the genera *Strebla* and *Euctenodes* by redescribing *Strebla vespertilionis* (F.) and *Euctenodes mirabilis*, Waterhouse, and to describe a new species in the later genus.

That these two genera are somewhat similar will be seen by the characters included in the combined description, but they may be differentiated by the length of the hind legs and the general stoutness of the body (Figs. 1 and 2). One of the chief reasons for the confusion of the two genera is that Waterhouse

[1] F. Muir (1912), Two New Species of *Ascodipteron*, *Bull. Mus. Compar. Zool. Harvard*, LIV, pp. 351–66, pl. 1–3 (see p. 356 and pl. 2, fig. 9).

[2] L. Falcoz (1923), Biospeologica, XLIX; Pupipara, *Arch. Zool. Exp.* LI, fascic. 4, pp. 522–52.

omitted the cross-veins from the wings in his figure of *Euctenodes mirabilis*, with the result that all specimens with cross-veins were placed in the genus *Strebla*, and that Speiser used the supposed absence of cross-veins in *Euctenodes* as a key-character separating that genus from *Strebla* in his work on STREBLIDAE[1].

The drawings for this study were made by Mr H. C. Gillings of the Molteno Institute. They were made in part from cleared mounts, and perhaps some unnecessary detail has been shown in certain structures between the palps and the antennae; some of these structures appear to be internal, and consequently cannot be seen in dried specimens.

Characteristics of the Subfamily Streblinae.

Head flattened and triangular in shape, with a ctenidium (Fig. 9) composed of very strong, closely set, black spines across the ventral surface, near the posterior margin, and continued up the sides for a short distance, so that its ends may be seen in dorsal view; this ctenidium strongly resembles that on the first ventral abdominal segment of the NYCTERIBIIDAE or on the dorsal surface of the head of a flea. The outline of the front of the head, including the palps, is rounded, and the head widens out behind, until at the base it is as wide as the thorax. *Thorax* with dorsal surface slightly convex, except at the sides where it is level; longer than broad, much wider dorsally than ventrally; at the sides there is a wide groove, in which the front legs lie when at rest (Fig. 9). There are four, long, strong bristles on the scutellum. *Tarsi* with terminal segment not much broader than the foregoing segments.

Other characters common to the two genera Euctenodes and Strebla.

The head is flattened; so, too, is the thorax to a lesser degree, and the abdomen is normal. *Head* with two large, more or less triangular chitinous plates (Figs. 3 *pl*, 5, 8) on the dorsal surface behind the eyes (Fig. 3 *e*) and antennae, separated from the posterior and lateral parts by pale, narrow, weakly chitinized areas; these plates may be readily located and will be used in explaining the position of the other parts; each plate differs somewhat in shape, and in the relations of the three smaller pieces of chitin which compose it, in the different species. A detailed description is given under *Euctenodes mirabilis*. *Antennae* (Figs. 3 *a*, 4, 6) situated in front of, and between, these triangular plates, lighter in colour than the surrounding parts, and composed of two visible segments and a plumose arista. An antenna which has been removed from the head and mounted in balsam appears to be three-segmented (as is normal in the section of the DIPTERA to which the STREBLIDAE belong), showing under a high power what is probably the real first segment in a very reduced condition (Fig. 6). What I shall, therefore, call the second segment is

[1] P. Speiser (1900), Ueber die Strebliden, Fledermausparasiten aus der Gruppe der Pupiparen Dipteren, *Arch. Naturg.* 66, I, pp. 31–70, pl. III, IV.

very large and encloses the third, except where the latter is exposed by a cleft in the dorso-lateral surface of the second (Fig. 4); it bears a strong curved bristle on the dorsal surface. The third segment is nearly spherical, and projects a little beyond the second, bearing a terminal arista, more or less plumose according to the species; its processes are branched several times, forming a compoundly plumose arista, in the same plane as the flattened head of the insect; branched processes have been noted by Muir in *Ascodipteron*, and also occur in the tsetse flies[1], but in these latter they lie in a vertical plane, while in the STREBLINAE the arista is turned so that its processes lie in a horizontal plane. In a balsam preparation, if a high power (1/6-inch objective) is focussed below the surface of the distal part of the second segment, a striking network may be seen, but I am unable to say whether it is on the inside of the hollow cup formed by the second, or on the third, segment; and in profile a series of tooth-like projections is visible on the inner side of the dorsal part of this cup (Fig. 6). *Palpi* (Fig. 3, *pa*) large, but hardly recognizable for what they are; their extent may be most readily determined from the ventral side, where they are covered with even transverse rows of short spines (Fig. 9, *pa*), while the adjacent parts of the head are not; the palpi are two leaf-like lobes, projecting from the front of the head, which turn up at the edges and bear numerous prominent spines. Because the tube of the proboscis, lying underneath in the middle line, reaches almost to the tip of the palpi, they frequently look as if they were fused, but a lighter line indicates the division between them; the spines, interlacing across the space between them and the head, also help to make the palpi look like part of the head. There is a fairly broad attachment with the head at the inner corner of each palp. *Posterior margin* of the head dorsally lobed, and bearing strong spines which are directed backward (Figs. 3, 5, 8). *Proboscis* composed of a basal, more or less globular part, and a rod-like distal part, which reaches almost to the tips of the palps (Fig. 9). The *thorax* has the dorsal transverse suture (Fig. 2, *s*) distinctly visible only at the sides. The scutellum is entirely free from bristles, except for the four long macrochaetae. The mesosternum is narrowed towards the anterior end, where it projects between the front coxae; there is a fairly deep emargination in its front margin; the suture between the meso- and metasterna passes behind the middle coxae, and forms an acute angle with the middle line (Fig. 9). The metasternum is produced to a blunt point behind, and to a point on each side behind the middle coxae, but is not quite so broad as the mesosternum. *Abdomen* with the first segment (Fig. 9) marked off ventrally by a series of spines along its posterior margin and having a triangular area (apex of triangle directed forward) covered with short spines; there are no spines on the rest of the segment, where the coxae rub against it.

[1] See Austen (1911), *A Handbook of the Tsetse-Flies*, Fig. 8, p. 12.

Principal differentiating characters.

Strebla: hind legs greatly elongated; general build less stout.
Euctenodes: hind legs not elongated; general build more stout.

Descriptions of Species.

Euctenodes mirabilis, C. O. Waterhouse.

Trans. Ent. Soc. London, 1879, p. 310, pl. x; Speiser, *Arch. Naturg.* 66. 1, 1900, pp. 63, 66.

(Figs. 2–4.)

Head: just in front of the antennae is a dark chitinous portion in the form of a broad Y (Fig. 3, *y*) with a short stem; each of the two chitinous plates (Fig. 3, *pl*) behind the antennae is closely applied at its anterior outer part to the eye, forming with the eye an irregular four-sided area, longer than broad, extending obliquely forwards and outwards, with corners rounded off; composed of three pieces, a piece nearest the antenna long, narrow and nearly parallel-sided, a median piece roughly triangular, with its apex almost reaching the front margin of the plate, and a minute, outer, wedge-shaped piece with its base immediately contiguous to the hind margin of the eye and its apex directed backwards; the anterior piece bears five short bristles as well as several smaller ones, the median triangular piece one bristle at its base and one at its apex, and the minute outer wedge one bristle at its middle; lateral parts of head consisting, on each side, of a minute chitinous anterior piece (Fig. 3, p^1) bearing several bristles, a larger chitinous piece (Fig. 3, p^2), closely bristled, extending back to the base of the ctenidial teeth, and, inside of and behind the extremity of the ctenidium, a roughly triangular piece (Fig. 3, p^3), with apex directed forward, divided by a median thickening into two portions, each of which bears one bristle; dorsal posterior margin of head four-lobed; on each side a broad outer lobe, irregularly wedge-shaped with its apex directed outwards and its posterior margin crenulated and bearing about seven strong bristles of varying length, as well as three finer, shorter bristles, lighter in colour, situated on the inner margin and directed backwards and towards the middle line; in the middle, two smaller lobes, each bearing one long, black bristle. *Antennae* (Figs. 3 *a*, 4) with a wide angle between them; arista plumose, the processes occurring on the outside down the greater part of the length, but on the inside only near the tip. *Eyes* (Fig. 3, *e*) with facettes not constant in number, sometimes nine, and in other examples seven; in the first case, six facettes lie in the form of a semi-circle, and three within it; when there are seven facettes, five form the semi-circle, and there are two within. *Thorax:* convex dorsal part covered sparsely with short, fine bristles, level lateral parts covered more thickly, and with longer bristles; about half-way between the normal transverse suture (Fig. 2, *s*) and the front margin of the thorax there are traces of another transverse suture (Fig. 2, s^1); sometimes

this is quite indistinct, and it is entirely lacking in *Euctenodes tonatiae*, sp. nov. and in *Strebla vespertilionis*. *Wings* short and broad; veins with uniform, very

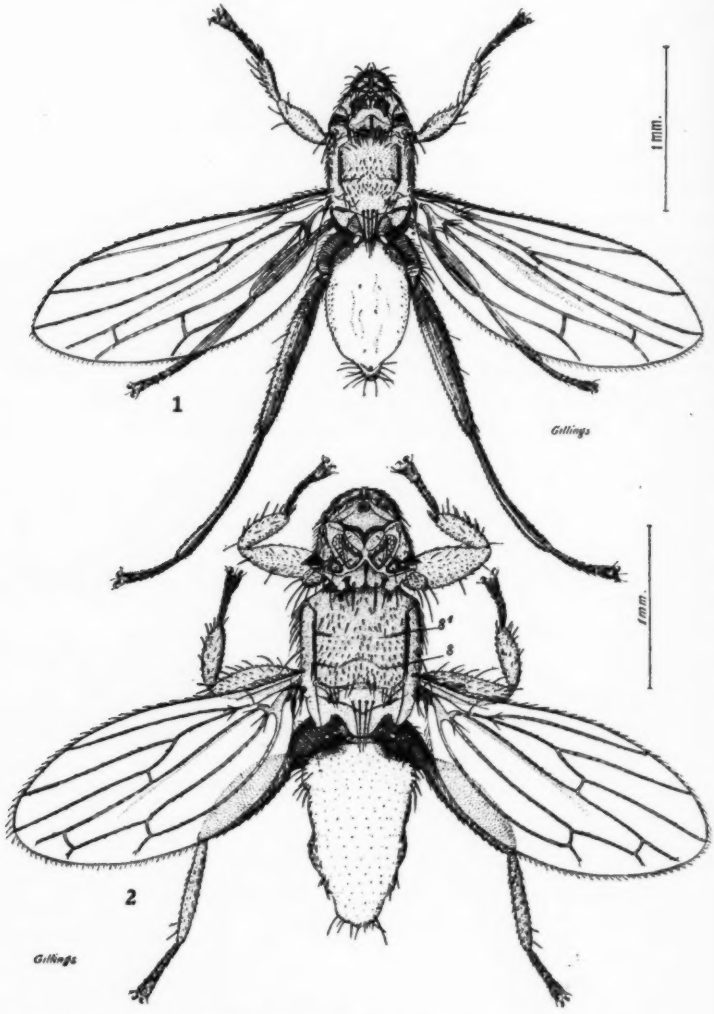

Fig. 1. *Strebla vespertilionis* (Fabr.), dorsal view.
Fig. 2. *Euctenodes mirabilis*, Waterhouse, dorsal view; *s*, normal transverse suture, distinct only at sides; s^1, indication of additional anterior transverse suture.

small spines. *Legs* normal. *Abdomen* laterally and ventrally bearing short spines evenly distributed; ventral basal chitinous plate with stronger spines on the posterior margin; a ring of spines encircles the abdomen ventrally and

laterally just in front of the small terminal segments. *Length* of body, 2·75 mm.; of wings, 2·2 mm.

Localities and hosts: the specimen from which I have made my redescription of Waterhouse's species is in spirit, from Pernambuco, collected off *Hemiderma perspicillatum*, L. (quoted as *brevicaudatum*) by W. A. Forbes (Brit. Mus., 1911–103); it has been carefully compared with Waterhouse's original type, a dried example from Colombia (host not recorded). Other specimens in spirit in the British Museum are (i) one from East Peru off *Phyllostomus hastatus*, Pall., collected by J. Kalinowski (1911–103); (ii) one from Yinyes, Bolivia, off the opossum *Glironia venusta*, Thos., received through Oldfield Thomas (1913–450); (iii) eight specimens off Vampires from Ocean Cave, south-west of San Lorenzo I. (1908–230); (iv) two examples off *Desmodus*, sp. from San Lorenzo I., off Callao, Peru (1913–450); (v) one specimen from Joinville, Humboldt, Santa Catharina State, Southern Brazil, collected by Wilh. Ehrhardt and received from N. C. Rothschild (1913–450); (vi) one from Humboldt, July, 1916, W. Ehrhardt, off *Phyllostomus* (1921–200). In the United States National Museum is a vial containing four examples off *Carollia perspicillatum aztecum* from Porto Bello, Panama, collected by R. C. Shannon, and one dried specimen from Paraiso, Panama Canal Zone, collected by A. Busck off "leaf-nosed short-tailed bat."

Euctenodes tonatiae, n. sp.

(Figs. 7–9.)

General appearance spiny, because the legs, besides being covered by a uniform coat of short spines, have numerous scattered, much longer, nearly erect spines. *Colour* paler than that of *Euctenodes mirabilis* or *Strebla vespertilionis*, and the bristles do not appear so black. *Head:* dorsal aspect showing all the parts which are present in *Euctenodes mirabilis*, but there are slight differences in shape and bristling which are best shown by comparing figures 3 and 8; the ctenidium forms a slight angle in the median ventral line, instead of a curve as in *Strebla vespertilionis* and *Euctenodes mirabilis*. *Antennae* at an angle with each other, the arista with processes about equally distributed on each side. *Eyes* with facettes very indistinct in the only specimen that I have, so that I am unable to make out their number, though the position of the group is clear. *Thorax:* convex dorsal part uniformly and fairly densely bristled; a little above the attachment of the wing on each side is a long bristle-hair; *Euctenodes mirabilis* also has a long bristle in this position; *Euctenodes tonatiae* lacks the anterior transverse suture noted under *Euctenodes mirabilis*. *Wings* short, broad and rounded; bristles on veins prominent, particularly one long bristle-hair at the apex of the wing at the end of the costa. *Legs* normal except for numerous, scattered, more or less erect bristles. *Abdomen:* dorsum entirely free from spines; sides thickly covered with spines, except where the hind legs rub on the abdomen; these lateral spines lie closely applied to the surface; spines not numerous on the under side, but uniformly dis-

tributed; *triangle* on first ventral segment not darkly chitinized. *Length* of body, 2·24 mm.; of wing, 1·39 mm.

Localities and hosts: the single specimen (*type*) from which I have made the description is in the British Museum, from Gualaguiza, Ecuador, off *Tonatia brasiliensis*, Peters, collected by E. Festa (1913–450).

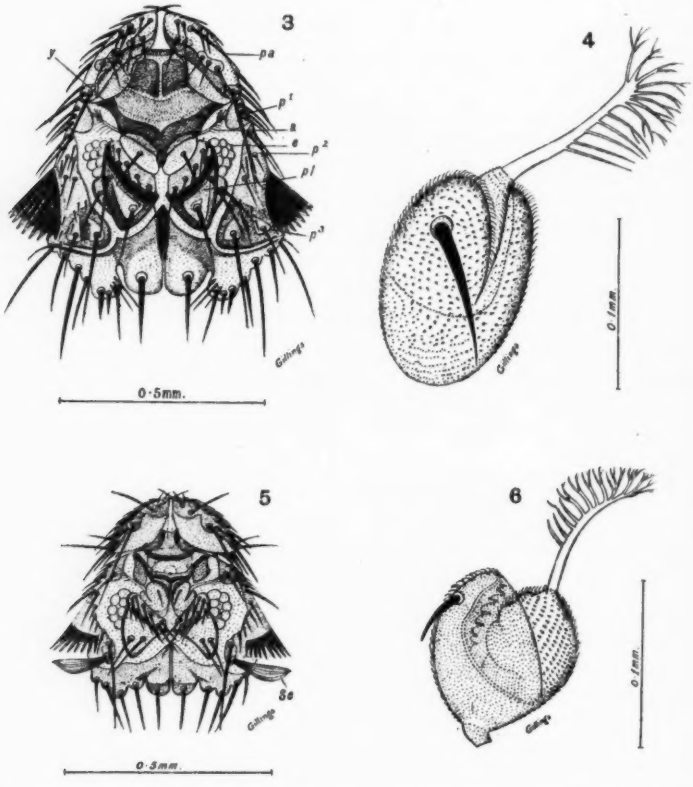

Fig. 3. *Euctenodes mirabilis*, head removed and mounted in balsam, dorsal view; *a*, arista of antenna; *e*, eye; *pa*, palp; *pl*, one of the two large chitinous plates, composed in this species of three pieces, behind the eyes and antennae; p^1, minute anterior lateral chitinous piece; p^2, larger second lateral chitinous piece; p^3, triangular posterior lateral chitinous piece; *y*, dark chitinous Y-shaped portion of head.

Fig. 4. *E. mirabilis*, antenna; 2nd and 3rd segments and arista, in dorsal view; a small part of the 3rd segment is visible through the dorso-lateral cleft in the 2nd, and the outline of the remainder of the 3rd is indicated by a dotted line.

Fig. 5. *Strebla vespertilionis*, head removed and mounted in balsam, dorsal view; *sc*, flattened and striated scale arising from posterior lateral chitinous piece.

Fig. 6. *S. vespertilionis*, antenna removed and mounted in balsam, in profile, showing minute basal segment, and 3rd segment partly covered by the cup-like 2nd segment.

Strebla vespertilionis (Fabr.).

Speiser, *Arch. Naturg.* 66. 1. 1900, pp. 38, 63, 65, pl. iv, figs. 1, 2[1].

(Figs. 1, 5, 6.)

Head (Fig. 5): compared with that of *Euctenodes mirabilis* and *E. tonatiae* its structure is strikingly different. Each *eye* appears much larger proportionately than in *E. mirabilis*, and each contains seven facettes, five arranged

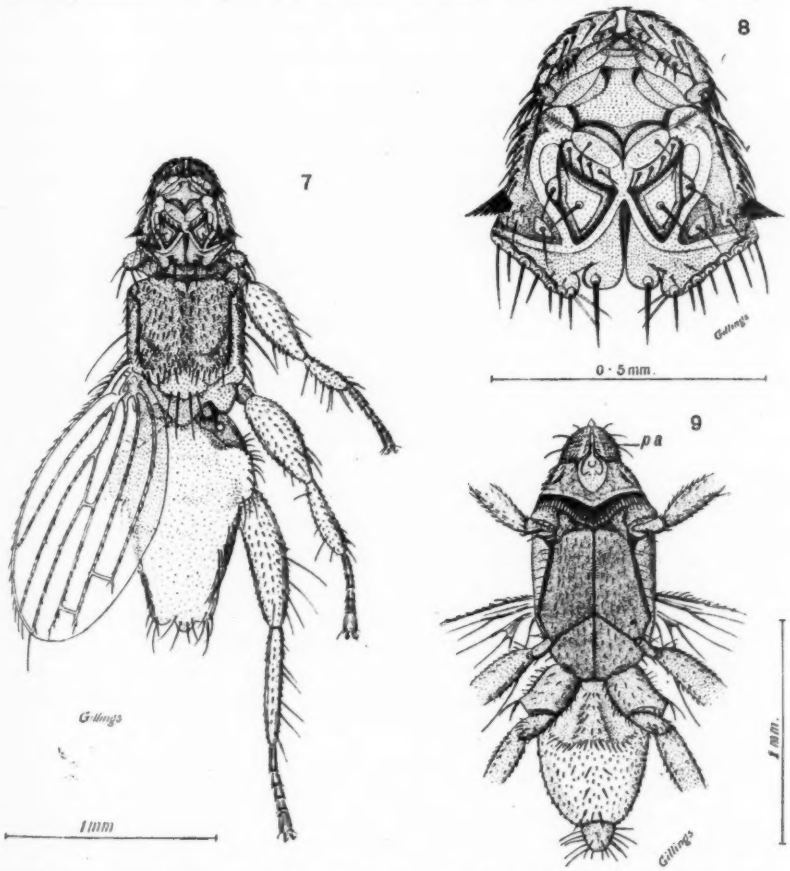

Fig. 7. *Euctenodes tonatiae*, n. sp., dorsal view. Fig. 8. *Euctenodes tonatiae*, enlarged dorsal view of head. Fig. 9. *Euctenodes tonatiae*, ventral view; *pa*, palp.

in a semi-circle and two within the half circle thus formed. The *antennae* appear bulging and prominent, and are almost continguous one with the other, thereby causing the median anterior piece of the head to have a much broader, more flattened, less **Y**-shaped form. The chitinous plates behind the antennae are different in shape and the minute outer wedge-shaped piece is absent.

[1] A full bibliography of this species is given by Speiser, *op. cit.* p. 63.

On each side of the head, the anterior lateral chitinous piece is larger and bears many bristles; the area behind this, extending to the base of the ctenidial teeth, is nearly bare, except for one or two bristles at its (anterior) apex and a few along its inner margin; and the posterior chitinous piece is more concealed by the inner extremity of the ctenidium, and bears two bristles, the posterior one of which is a long, broad, flattened, outwardly-directed scale (Fig. 5, *sc*), evidently a modified bristle of extraordinary form, seen in a balsam preparation under a high power (1/6-inch objective) to be longitudinally striated, and recalling strongly the fin of a fish; this scale appears as an ordinary spine from the dorsal aspect, when only its narrow upper edge is seen, and the head must be removed and mounted for its proper examination. The form of the posterior marginal lobes is strikingly different as will be seen by comparing Figs. 3 and 5; each outer lobe has only four bristles, borne on strong marginal crenulations. *Thorax:* convex dorsal part much more sparsely bristled than in *E. tonatiae* and *E. mirabilis*; the anterior transverse suture occurring in *E. mirabilis* is absent. *Wings* long and comparatively slender; 1st, 4th and 5th longitudinal veins practically free from bristles, the others with sparse short bristles; cross-vein between the 5th and 6th longitudinal veins inclined slightly outward at the bottom; cross-vein between the 3rd and 4th veins weak in the middle and situated nearer the branching of the 3rd vein than in *E. tonatiae* or *E. mirabilis*. *Legs:* first pair of normal length; front coxae without transverse rows of short spines; middle pair somewhat lengthened; hind legs strikingly elongated, about twice as long as the front pair; femur reaching beyond the last cross-vein of the wings, the wings reaching just beyond the middle of the tibia; first segment of the tarsus elongated, and the same length as the terminal segment, or as the three intermediate segments together. *Abdomen:* no long bristles dorsally, except on the first segment and at the tip; ventrally, between the first segment and the apex, are three pairs of bristles, which seem to mark off segments not otherwise usually distinguishable; sometimes six segments are distinctly visible; dorsum free from short bristles, but remainder of abdomen uniformly covered with small bristles. *Length* of body, 2·09 mm.; of wings, 2·12 mm.

Localities and hosts: there are eight specimens in spirit, and the parts of two, mounted in balsam, in the British Museum. They are from Joinville, Humboldt, Santa Catharina State, Southern Brazil, 5. xi. 1912 (Wilh. Ehrhardt, 1913–450), collected off *Lonchoglossa caudifera*, Geoffr. (*Glossophaga ecaudata*). Speiser (*op. cit.* p. 63) gives a list of records including Colombia and other parts of South America, Jamaica and Mexico, and naming as hosts *Vampyrops lineatus*, E. Geoffr., and *Lonchoglossa caudifera*, Geoffr. (*ecaudata*).

Speiser's work has been followed in referring this material to the species *vespertilionis*, Fabr.; there has been no opportunity to compare it with the actual type of Fabricius, which (according to information received through Mr F. W. Edwards) is not at Copenhagen, but may be still preserved at Kiel.

THE RANGE OF VARIATION OF *HYMENOLEPIS NANA* IN RATS AND MICE.

By H. A. BAYLIS, M.A., D.Sc.,

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Dr Woodland kindly submitted to me the majority of the worms recovered by him on dissecting the mice used in his very interesting experiment (see his paper that follows[1]), with a request for my opinion as to the identity of the species. The material, which was handed to me preserved in formalin, consisted only in part of complete specimens, a number of the worms having been broken up during collection. I stained most of the whole specimens with dilute haematoxylin and mounted them in Canada balsam. Upon microscopic examination it became evident that the specimens were of various ages and degrees of maturity, and one specimen was of exceptionally large size. This individual, in a fairly contracted condition, measures (after mounting and without stretching) 36 mm. in length, and has about 650 recognisable segments. Mature segments (*i.e.* segments with testes and ovary both fully developed) begin to appear at about the 320th segment from the scolex, and gravid segments at about the 490th. There are thus about 160 gravid segments. It will be evident from these details that this specimen agrees with my description of "*Hymenolepis longior*[2]." Two of the young forms among Dr Woodland's material also seemed to agree with "*H. longior*" rather than with what I had regarded as *H. nana*.

The presence of these anomalous specimens at once raised a difficulty. If, as Dr Woodland believed, all the worms found in the mice were developed from the eggs administered by him in the course of the experiment[3], then either "*H. longior*" must occur in Man, or it could not be regarded as a "good" species. Dr Woodland was very strongly in favour of the latter view, and after going more fully into the matter, and investigating, with his assistance, the amount of variation occurring in considerable series of specimens, I now agree with him that "*H. longior*," as a distinct species, cannot be maintained, but must be regarded as a synonym of *H. nana*.

[1] *Parasitology*, XVI. p. 424.

[2] *Parasitology*, XIV. p. 2 (1922).

[3] Dr Woodland has stated his conviction that ectoparasites are negligible as a source of infection, and he accordingly took no precautions against them. Although it has been shown by various experimenters that the development of *H. nana* and of "*H. longior*" can take place directly, without the aid of an intermediate host, it does not seem to follow that ectoparasites never act as intermediate hosts for the worms, and the possibility of accidental infection by this means should not be overlooked.

The supposed distinctions between *H. nana fraterna* and "*H. longior*," as tabulated in my former paper, rested partly upon figures which were actually erroneous, and partly upon the fact that my specimens appeared to fall into two quite distinct size-groups. No other conclusion seemed possible at the time than that these belonged to two distinct, though extremely closely related, forms.

The erroneous figures in the table referred to are those relating to the eggs of *H. nana fraterna*. For these, owing to difficulties met with in obtaining satisfactory measurements from preserved specimens, I was induced to rely upon measurements taken from living eggs by Mr Dudgeon and Dr Stevenson. These eggs, as Dr Woodland suggested to me, must have been those of another species, and not of *H. nana*[1], since Dr Woodland has not met with any constant differences, in size or shape, among the eggs of *H. nana* and of "*H. longior*." Moreover, I have since carefully examined eggs teased from gravid segments of my original specimens of "*H. longior*," and similar eggs from *H. nana fraterna*, and have been unable to find any such differences between them. The eggs were mounted in lactophenol, which prevents the shrinkage generally caused in the membranes by other reagents. All the measurements obtained by this method were in general agreement with those given in my paper for "*H. longior*" and with those obtained by Dr Woodland for the living eggs, while the inner shell was in all cases more or less lemon-shaped and provided with polar knobs, and in favourable specimens polar filaments could generally be detected.

This apparently important differential character of "*H. longior*" being thus removed, there remained other characters depending upon the general dimensions of the worms and upon the number and disposition of segments. Mere length, of course, by itself, was of no value, since, even apart from the question of degree of contraction, the extreme sizes of the two supposed forms were known to overlap. The total number of segments, however, and the points in the series at which mature and gravid segments began, could not be ignored as specific characters until a special study had been made of the amount of variation in these particulars.

In the table in my former paper it was stated that mature segments appeared at, roughly, the 75th segment in *H. nana fraterna* and at, roughly, the 250th in "*H. longior*," while there was a corresponding difference in the points at which the segments became gravid. It seemed essential, therefore, to discover whether the apparently large gap between these figures could be filled up by intermediate forms if the segments of a sufficiently large series of specimens were counted. I have therefore made a series of counts from specimens selected on a general size basis from a long series stained and mounted by Dr Woodland for the purpose, the lengths of which, as measured in balsam, range from 9 mm. to 51 mm. The results of these counts are given in the table below. It is, of course, obvious that the figures can only be

[1] The measurements agree closely with those of the eggs of *H. diminuta* (Rud.).

approximate where the changes from immature to mature, and from mature to gravid, segments are so gradual, and where the young segments near the scolex are so ill-defined. Nevertheless, they possess a certain value for comparison among themselves.

Specimens of Hymenolepis nana *derived from several naturally-infected laboratory mice*.*

Total number of segments (approx.)	Approx. length in mm. (in balsam)	Mature segments begin at (roughly)	Gravid segments begin at (roughly)	Number of gravid segments
135	9	70th	105th	30
185	11	35th	110th	75
230	13	100th	140th	90
230	15	110th	150th	80
245	14	95th	165th	80
290	13·5	80th	190th	100
310	16	150th	245th	65
350	17·5	175th	260th	90
360	18	100th	210th	150
410	19	250th	340th	70
460	23	150th	275th	185
475	28	150th	260th	110
495	20	250th	350th	145
505	26	180th	300th	205
510	21·5	200th	320th	190
565	24	250th	375th	190
565	31	200th	325th	240
660	34·5	300th	450th	210
840	51	450th	600th	240

* The specimens are arranged in ascending order of approximate total number of segments, the measurements of length, which are subject to great alteration by contraction, being considered of secondary importance.

The tabulated figures represent nineteen specimens, selected from several sets which together gave a complete range of sizes varying from 7 mm. to 80 mm., according to Dr Woodland's measurements. It is evident that they form a fairly regular series, showing (1) that the total number of segments is fairly closely correlated with the length of the worm and (2) that as the length and the total number of segments increase, the number of segments in each distinguishable region of the body—immature, mature and gravid—increases at the same time in a fairly regular manner. There is no clear gap in the figures, such as would indicate that we are concerned with more than one species or race. It seems a reasonable inference, therefore, that all the specimens belong to a single species having a very wide range of variation in size and in number of segments.

Other supposed differences between *H. nana* and "*H. longior*" (diameters of scolex and suckers, length of rostellar hooks) are very slight, and presumably represent no more than individual variations. It may be concluded, therefore, that *H. nana*, as it occurs in mice and rats, is a species with an exceptionally wide range of variation among mature individuals. In my former paper it was remarked that "the discrepancies in the measurements of

total length given by different writers for '*H. murina*' are greater than could readily be accounted for by different states of contraction in complete individuals of the same species." This is undoubtedly true, but the real explanation of these discrepancies appears to be that the growth of the individual may proceed almost indefinitely, the number of segments being increased all the time, in such hosts as the rat and mouse. Whether the same range of size occurs in *H. nana* in Man is at present uncertain. The evidence so far available seems to indicate that the human worms always remain small, which may possibly mean that the rat and mouse are the hosts to which the species is best adapted.

(*MS. received for publication* 19. VIII. 1924.—Ed.)

THE FILARIID OF THE CAMEL, *ACANTHOCHEILONEMA EVANSI* (LEWIS).

By C. L. BOULENGER, M.A., D.Sc.,

Professor of Zoology, Bedford College, University of London.

(With 4 Text-figures.)

Although the common Filariid parasite of the Camel was identified as a distinct species more than forty years ago (Lewis, 1882), the recent memoir on Indian Nematodes by Baylis and Daubney (1923) contains the first intelligible account of the structure of the adult worm. Their description of the species, which they refer to the genus *Acanthocheilonema*, is based partly on some rather fragmentary material received from the Zoological Survey of India, and partly on notes and drawings supplied by Major F. H. Stewart, I.M.S.

Whilst in the Punjab in 1921 I had the opportunity of examining a number of well-preserved specimens from the collection of the Lahore Veterinary College. I am, therefore, in a position to supplement the account given by Baylis and Daubney and to correct some errors due, no doubt, to the poor state of preservation of the material at their disposal. My interpretation of certain structures differs, moreover, from that adopted by these authors and helps, I think, in estimating the systematic position of this interesting Filariid.

The description in this paper is based entirely on the notes and *camera-lucida* drawings made by me in India. As explained below, I accept, provisionally, the position assigned to the parasite in the genus *Acanthocheilonema* Cobbold.

Acanthocheilonema evansi (Lewis, 1882).

Filaria evansi Lewis, 1882.

Female. The female worms measure 170 to 215 mm. in length, with a maximum thickness of 0·55 to 0·65 mm. The body tapers from the middle about equally to the two extremities, anterior and posterior ends being difficult to distinguish under low magnifications, as both appear truncate and of approximately equal diameter.

The head has a maximum breadth of 0·12 to 0·14 mm.; it appears almost oblong when viewed *en face*, owing to the formation of four somewhat laterally directed prominences which bear the submedian papillae; the latter are double, there being 10 head-papillae in all, 2 lateral and 8 submedian. Owing to the position of the head prominences the anterior extremity of the body appears broad and truncate in dorsal or ventral aspect (Text-fig. 1, *A*), narrow and rounded in a lateral view (Text-fig. 1, *B*).

The oesophagus is long and divided into anterior muscular and posterior glandular sections, the two regions measuring 0·75 to 0·8 mm. and 5·5 to 5·8 mm. in length, respectively. These measurements differ markedly from those

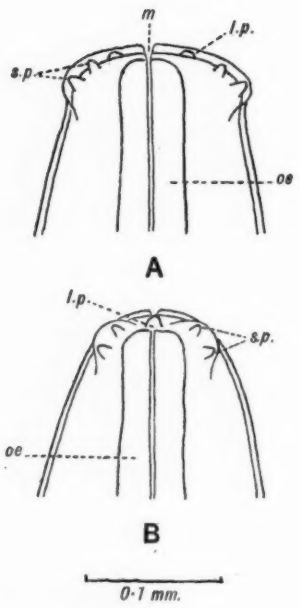

Fig. 1.

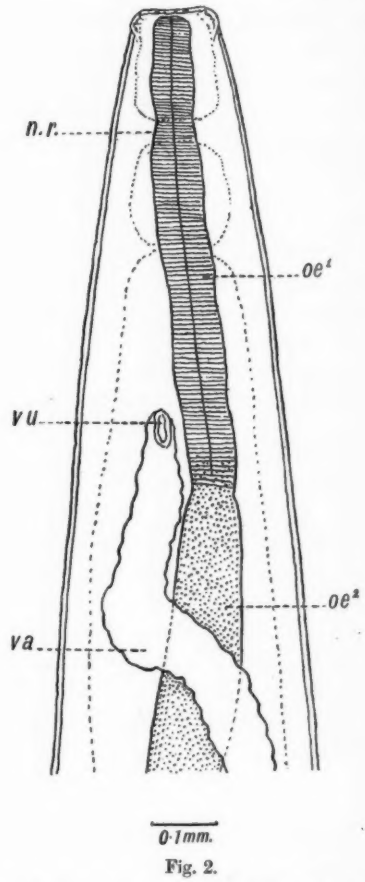

Fig. 2.

Fig. 1. *Acanthocheilonema evansi.* Head-region: *A.* dorsal view; *B.* lateral view; *l.p.* lateral papilla; *m.* mouth; *oe.* oesophagus; *s.p.* submedian papilla.

Fig. 2. *Acanthocheilonema evansi.* Anterior extremity of female, ventral view: *n.r.* nerve-ring; oe^1. anterior section of oesophagus; oe^2. posterior section of oesophagus; *va.* vagina; *vu.* vulva.

recorded by Baylis and Daubney; it is evident, however, from their figure (reproduced from a drawing by Major Stewart) that a slight constriction of the anterior oesophagus at the level of the nerve-ring has been taken to mark the termination of that organ; the junction of the two regions proves to be considerably further back in the body (Text-fig. 2).

The anus is situated 0·25 to 0·3 mm. from the posterior end; the tail terminates bluntly and is most characteristic, being thickened and provided with a pair of very short, broad processes about 0·05 mm. from the extremity (Text-fig. 3). The presence of these short caudal appendages makes the tail appear obliquely truncate when seen in side view under low magnifications (Text-fig. 3, *B*). Major Stewart's notes refer to "three faint grooves" on the posterior border of the tail; the single female examined by Baylis and Daubney was evidently badly preserved, they are, however, correct in considering that these grooves indicate the position of caudal appendages similar to those present in other species of *Acanthocheilonema*.

The vulva (Text-fig. 2) is a small, inconspicuous aperture situated 0·57 to 0·65 mm. from the anterior extremity of the body; it leads into a long, narrow

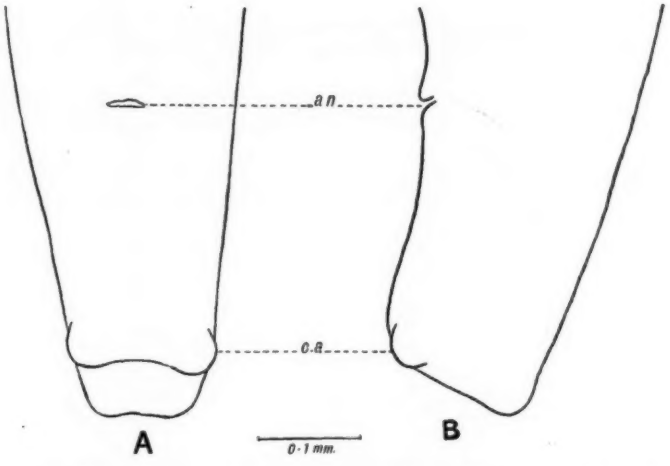

Fig. 3. *Acanthocheilonema evansi*. Posterior extremity of female: *A*, ventral view; *B*, lateral view; *an*, anus; *c.a*, "caudal appendage."

vagina, directed posteriorly. The worm is ovo-viviparous, the uterus being packed with tiny embryos.

Males. The male worms are considerably smaller than the females, the specimens examined measuring 75 to 90 mm. in length with a maximum thickness of 0·25 to 0·29 mm.

The body tapers gradually from the middle to the anterior end, the head having a breadth of 0·1 to 0·11 mm.; the posterior extremity, unlike that of the female, is much attenuated and coiled in a loose spiral.

The oesophagus resembles that of the female; it is, however, shorter, the anterior and posterior regions measuring 0·55 mm. and 4·7 mm. respectively.

The cloacal opening is situated 0·075 to 0·1 mm. from the posterior end, the tail has a rounded extremity (Text-fig. 4) and is without lateral alae.

Four pairs of preanal and four pairs of postanal papillae are present, the last preanal and the first postanal papillae being close to the cloacal aperture, the latter are rather more laterally situated than the other papillae and are sometimes very inconspicuous; they do not appear in the figure which Baylis and Daubney reproduce from Major Stewart's drawing.

The spicules are dissimilar and markedly unequal in length; the short spicule is thick and approximately conical in shape, with a length of 0·175 to 0·19 mm., the long spicule measures 0·75 to 0·9 mm. in length and, as described

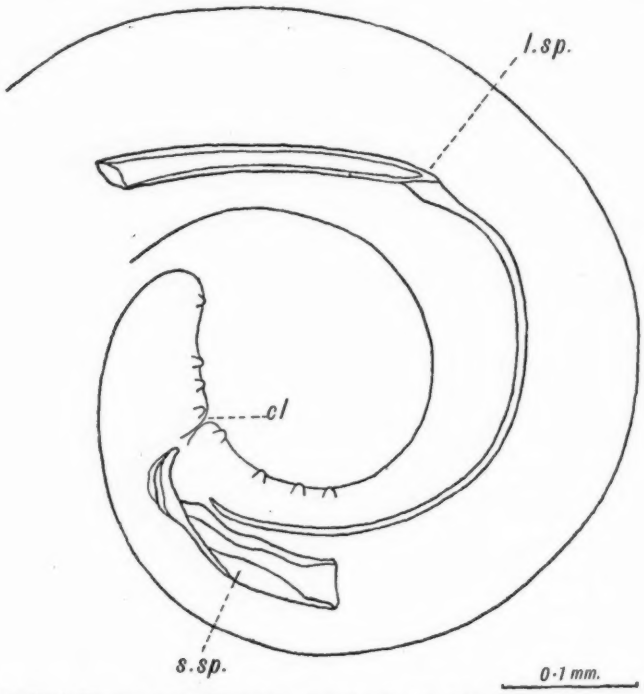

Fig. 4. *Acanthocheilonema evansi.* Posterior extremity of male, lateral view: *cl.* cloacal opening; *l.sp.* long spicule; *s.sp.* short spicule.

by Baylis and Daubney, consists of a thick proximal portion and a much thinner distal portion which is largely membranous (Text-fig. 4).

Systematic position. The observations recorded in this paper tend to confirm the view held by Baylis and Daubney as to the relationship of the camel Filariid; in the shape of the head and the characters of the two sections of the oesophagus "*Filaria*" *evansi* strongly resembles the various forms which have been included in Cobbold's genus *Acanthocheilonema* since it was redefined by Railliet, Henry and Langeron (1912); the short, blunt processes close to the tip of the tail in the females are, moreover, undoubtedly homologous with the

"caudal appendages" which are such a characteristic feature of these species. Such caudal appendages are apparently absent in the male and in this sex the posterior extremity of the body, with its short, rounded tail and four pairs of postanal papillae, recalls that found in the genus *Filaria* s.str. rather than in *Acanthocheilonema*. Baylis and Daubney, however, state that they have noted an additional pair of postanal papillae in *A. gracile*; it seems to me also possible that the last small pair of postanal papillae in the species from the camel (Text-fig. 4) may really represent the caudal appendages of other forms; "*Filaria*" *evansi* may therefore, provisionally, be retained in the genus *Acanthocheilonema*, as a somewhat aberrant species characterised particularly by the short, thick tail in the two sexes.

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ON THE DEVELOPMENT OF THE HUMAN *HYMENOLEPIS NANA* (SIEBOLD 1852) IN THE WHITE MOUSE; WITH REMARKS ON "*H. FRATERNA*," "*H. LONGIOR*" AND *H. DIMINUTA*.

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Historical.

Joyeux, in his well-known memoir (1920), gives an account of three experiments in which he fed rats and mice on fresh eggs of *Hymenolepis nana* obtained from decanted or centrifuged human stools, and comes to the conclusion that these rodents are uninfectable. This account was unsatisfactory from several standpoints, as also were the experiments, as indeed Joyeux in part admits. The ages or sizes of the rats and mice were not specified, nor the exact manner in which the animals were made to ingest the *H. nana* eggs, nor the nature of the food. The twenty-two rats and mice employed were not previously ascertained to be free from infection with the "*Hymenolepis fraterna*" common to these animals, and in the second and third experiments the animals were fed with eggs both of the human *H. nana* and of the murine "*H. fraterna*," thus confusing the results which were obtained, since, as I have recently shown (Woodland, 1924 *a*), delayed development of ingested eggs is not uncommon in mice. Joyeux also relates experiments by Schnell, who fed two mice on *H. nana* worms from an autopsy, and by Malvoz, who fed some white rats and mice on bread mixed with faeces containing *H. nana* eggs, in all of which the results were negative.

In 1916 Stewart fed two young white rats (about forty-two days old) on *H. nana* eggs from an Indian soldier with negative results; possibly due to previous medication of the patient with oil of chenopodium. In 1922 Chandler also fed two white rats with living *H. nana* eggs with similar results, and Scott (1923) succeeded no better on feeding two mice with stale *H. nana* eggs.

The foregoing evidence, unsatisfactory as it is in individual experiments, is yet in the aggregate of some weight, since it proves that, under certain conditions, infection of rats and mice with *H. nana* eggs is unquestionably difficult.

Evidence in support of the opposite conclusion is, however, afforded by the two papers of Saeki (1920) and Uchimura (1922), published in Japanese and

inaccessible in London. The contents of these two papers are only known to English writers from abstracts published in the *Tropical Diseases Bulletin* and the *Japan Medical World*, and in consequence many details of procedure are lacking. Saeki obtained a plentiful supply of *H. nana* eggs from a girl of nine years and fed an unstated number of mice and albino and house rats with "in most cases" positive results. The details provided of the development of the worm are similar to those already described for the development of "*H. fraterna*" in these animals. Uchimura confirmed Saeki as regards the early stages of development.

The foregoing summary of conflicting conclusions showed that further enquiry into the subject was desirable and I gladly availed myself of the kind hospitality of Dr A. Bettencourt this last spring to put the matter once more to experimental test on a large scale at Lisbon, where *H. nana* infection is common among children (Bettencourt, 1916; Paes, 1917).

THE OBTAINMENT OF *HYMENOLEPIS*-FREE MICE.

Previously to visiting Portugal it was necessary to obtain a large number of mice known for certain to be free from "*Hymenolepis fraterna*," and on January 1st of this year I commenced the process of examining in all 228 very young mice (most of them well under two months old) and gradually eliminating all those infected with "*H. fraterna*." It is necessary for me to state some of the details of this process in order to convince others that in the end the 57 mice with which I conducted the experiment were really free from "*H. fraterna*."

The first batch of 48 mice examined (Series A) proved to be heavily infected—34 out of the 48 eliminated after four examinations of their faeces—and after rejecting 10 more for acute coccidiosis at the first examination and 3 having died, I only had 1 survivor! In the second batch of 23 young mice (Series B), 5 only, after two examinations, were found to be infected, but 7 having died and 3 being rejected at the first examination for coccidiosis, only 8 survivors remained. In the third or C Series of 49 mice (derived from the same bad source as the A Series) 34 were found to be infected after two examinations, 7 died and 3 were rejected for coccidiosis at the first examination, leaving 6 survivors. In the fourth, or D Series, of 48 mice (derived from the same source as the B Series) 13 were found to be infected after four examinations, 6 died, 3 were rejected for coccidiosis at the first examination, 1 was rejected because it escaped from its cage for a couple of hours, 2 were rejected on account of skin disease and 1 for lung trouble, leaving 22 survivors. In the fifth, or E Series, of 48 mice (obtained from the same source as Series A and C), 24 were found to be infected after three examinations, 3 died, 6 were rejected on account of coccidiosis at the first examination, 5 were rejected as having been temporarily caged with mice which afterwards showed infection and therefore under suspicion (2 of these 5 subsequently became infected), and 1 escaped in Lisbon, leaving 9 survivors. In the sixth or F Series of 12 pure albino mice, obtained from a new and exceptionally clean source, not one showed infection, and all, except one which died, were used in the final experiment.

Thus, out of a total of 228 mice examined from January 1st onwards, 112 proved to be infected with "*H. fraterna*": good evidence for Joyeux's statement that young mice are especially prone to infection; and the fact that the

degree of infection among the several batches of mice evidently depends upon the length of time uninfected mice have been herded with infected mice is also good additional evidence for the one-host mode of transmission of the parasite, if such were needed. Of the 57 survivors, none showed infection after the fourth examination (when the "youngest" mice examined were twenty days old, *i.e.* twenty days had elapsed since the first examination, and the "oldest" thirty days "old"). The fifth examination of all the five series of mice was held on February 20th–22nd, the sixth February 25th–29th, and the seventh examination on March 5th–7th, when the "youngest" mice (the 11 "clean" mice of the F Series) were thirty-seven days "old," the next "youngest" forty-five days "old," and the "oldest" sixty-one days "old." Finally, to obviate all possibility of the supposition that any mice still harboured "*H. fraterna*," I conducted an eighth and final faecal examination of the 30 mice egg-fed with *H. nana* eggs after I had arrived in Lisbon. This final examination was made in all cases four days after the egg-feeding and therefore well before there was any possibility of *H. nana* eggs appearing in the faeces. Ten mice were fed on *H. nana* eggs on March 25th and were examined faecally on March 29th (when the "youngest" of the mice were fifty-nine days "old" and the "oldest" seventy-seven days), 14 mice were egg-fed on March 28th and examined faecally on April 1st (when the "youngest"—mouse F2—was sixty-one days "old," the next "youngest" sixty-nine days and the "oldest" eighty-nine days), and 7 mice were egg-fed on March 31st and examined faecally on April 4th (when the "youngest"—5 of the F Series—were sixty-five days "old" and the other 2 eighty-one and eighty-four days "old"). In all cases the faeces were entirely free from eggs. In view of this fact that the faeces of the "youngest" mice (F Series) were proved to have been free from eggs for a period of fifty-nine days and the next "youngest" (E Series) for sixty-eight days and the remainder for still longer periods, and the further fact that when all the mice were killed a week or so after the eighth examination, not only the 27 mice used as controls but also 23 of the 30 mice fed on *H. nana* eggs, *i.e.* 50 mice out of the 57, were found to be entirely free from *Hymenolepis* infection, I think I am justified in concluding that the 7 egg-fed mice which were found to contain *Hymenolepis* were also free from "*H. fraterna*" infection and that the *Hymenolepis* found were wholly derived from the *H. nana* eggs swallowed. This conclusion is confirmed by the further significant fact that the *H. nana* worms found in the 7 mice were all, as it chanced, of sizes more or less appropriate to the lengths of time which had elapsed since the egg-feeding.

I need not recapitulate in detail all the precautions taken in regard to the feeding and general care of the mice, since these were on the whole identical with those I have described at length in my previous paper on the life-cycle of "*H. fraterna*" (Woodland, 1924 *a*). It is necessary and important to mention, however, that on this occasion all the mice which were egg-fed on *H. nana* eggs were kept isolated after the egg-feeding and that all mice were kept

isolated during the period of the first seven faecal examinations (save that a number of the E Series and a few of the D Series were temporarily caged together in pairs or threes between the first and second examinations), though subsequent to this period they were paired and placed in new specially-constructed cages for about a week while being transported to Lisbon on the boat. On arrival in Lisbon the mice to be treated with *H. nana* eggs were again all isolated, but the 27 control mice were allowed to remain paired.

Another important difference of procedure I must mention, since it may have proved to be a considerable factor in the results obtained, is that from the first I fed the mice on carefully-washed cabbage or lettuce as well as bread, occasional cheese and milk on alternate days. The result of this Vitamin B diet was that all the mice, though very small when I commenced the work, were full-grown adults at the period of egg-feeding and not half-grown like many of the mice which I employed on the previous occasion (Woodland, 1924 *a*). I shall refer to this fact again when discussing the results I obtained.

One other difference of procedure it may be necessary to mention is that on this occasion I did not search the mice for fleas or other ecto-parasites since they have already been proved (Joyeux, 1920, pp. 148–9; Woodland, 1924 *a*) to be without significance. In my previous work I mentioned that among 63 mice specially examined I obtained only two fleas, and since on no occasion did I observe fleas on the 228 young mice employed in the present work (and the 57 mice with which I finally experimented were frequently handled), I think it is fair to conclude that the fleas were not more abundant than on the previous occasion and may be regarded as negligible.

THE INGESTION OF *H. NANA* EGGS BY 30 OUT OF THE 57 MICE AND THE RESULTS OBTAINED.

On arriving in Lisbon I selected at random 30 of the 57 mice and isolated these in separate cages. On March 25th I was provided with fresh samples of faeces of four children (not previously medicated), one of which was heavily infected with *H. nana* eggs. This sample I mixed with a small quantity of water to a thick-soup consistency and found that every thin smear on a slide contained on an average four or five eggs. Though I examined many of these eggs on a warm stage, treating some with dilute HCl acid, yet I could detect no movements in the embryos; nevertheless the eggs were undoubtedly fresh[1] and therefore suitable for my purpose. I thereupon squirted down the throats of 10 mice quantities of about 1 c.c. of this faecal mixture, most of which were seen to be swallowed. Each mouse must thus have ingested at least twenty to fifty eggs. At the same time I diluted the remaining faecal soup, strained it through thin silk gauze and centrifuged, and then plentifully sprinkled with the deposit (containing great numbers of eggs) ten small cubes of bread previously soaked in milk. Each such cube was the only food each

[1] Fresh eggs of "*H. fraterna*" are also usually immobile. Chandler (1922) says that live embryos show movements if the egg-envelopes be torn.

of these 10 mice received on this date, and next morning the whole of the bread was found to have been eaten, except in one case, in which half the bread had been left. On examining these 10 mice on April 7th and 8th (thirteen and fourteen days after egg-feeding) by the process of slitting open successive lengths of the last 12–14 cm. of the small intestine, scraping with a scalpel and mounting the scrapings in water on slides, only 2 of the 10 mice were found to be infected. In a mouse labelled D31, four immature young worms only (the longest about 6 mm.[1] and the others 3–4 mm.) were found in the last 5 cm. of the intestine. In another mouse—E20—one young worm about 10 mm. long was found in the last 6 cm. of the intestine, and four more (two about 8 mm. long and the others 4–5 mm.) in the next 6 cm.

On March 28th I again received perfectly fresh richly-infected stools from a child and in this sample I detected vigorous movements of the embryos in at least two of the eggs. This sample only differed from the previous sample used in that it had been placed for an hour in an incubator at body temperature. With this material I fed 13 more mice in the manner already described. All of these mice ate the cubes of bread provided. On April 15th (eighteen days after the egg-feeding) I killed 12 of these mice and found 3 to be infected. In D14, in the last 8 cm. of the intestine, I found two worms, one well over and one about 20 mm. in length, with their hind proglottids full of eggs, and *eggs were also present in the faeces of the mouse.* Eggs can thus appear in the faeces on the eighteenth day after infection (Saeki says sixteen to seventeen days). In A19, in the last 8 cm. of the intestine, I found one worm fully 20 mm. long, one smaller worm about 8–10 mm. long, one about 7 or 8 mm. long and one small worm about 3 mm. long, and in the next 8 cm. of the intestine I found a piece (about 7 mm. long) of a large worm. The faeces of this mouse contained no eggs. In the third mouse—B3—I found in the last 8 cm. of the intestine one worm about 12–13 mm. long, one about 8 mm. long, one about 6 mm. and one fragment of a worm (nearly mature) 4 mm. long. The faeces contained no eggs. On April 23rd (twenty-six days after egg-feeding) I killed the last (thirteenth) of this second batch of mice and found in the last 7 cm. of the intestine three young worms, the longest about 7 mm. Thus out of these 13 mice 4 were found to be infected.

On March 31st I fed 7 more mice with the same rich faecal material used on the previous occasion—now three days old. Though I could detect no movements of the embryos in the undiluted stool, yet on centrifuging it I found several very active embryos. Four of the mice were fed in the usual way, but the other 3 ingested quantities of the centrifuged deposit, and all of them ate the cubes of bread. I killed these 7 mice on April 17th (seventeen days after egg-feeding) and found only one to be infected. This mouse—D21—was one of the three fed on centrifuged faecal deposit and in the last 9 cm. of the intestine I found four mature worms, about 15–20 mm. long, with eggs in the hind proglottids, and five smaller worms, 5–6 mm. long. The faeces contained no eggs.

[1] These and the following worm-lengths are all very approximate.

CONSIDERATION OF THE FOREGOING RESULTS.

23·3 per cent. only of the 30 egg-fed mice thus became infected, though each mouse must have swallowed at least thirty to forty eggs and probably more. This percentage is very low when compared with the 90 per cent. infection which I obtained on feeding mice with "*H. fraterna*" eggs (Woodland, 1924 *a*). The difference may be due to one or more of three factors. One factor may be change of host. Eggs which have developed in the human subject for many generations may be adversely affected when introduced into the mouse. Another factor may be the size of the mice employed. In my former work on "*H. fraterna*" the 18 mice out of 20 which became infected averaged 19·2 gm. in weight (minimum 6 gm. and maximum 26·1 gm.), while the mice employed on the present occasion were all full-grown adults and (though I did not weigh them) must have averaged nearly 30 gm. in weight. I may also call to mind that when, in my previous work, I tried to infect 5 large mice (of an average weight of 31·4 gm.) with "*H. fraterna*" eggs, I only succeeded in infecting 1 (20 per cent.!), though it is true that the same faecal material was used to infect 5 *young* rats and only 2 became infected (change of host?). It is, however, well known that both *H. nana* and "*H. fraterna*" develop most easily in young individuals and the fact of my mice being full grown may have accounted for the relatively low percentage of infection.

Another factor may have been the well-nourished condition of the mice used on the present occasion. In my "*H. fraterna*" experiments all the mice had been without vegetable food until three days before they were fed on the "*H. fraterna*" eggs, *i.e.* for periods between thirty-three and fifty-five days, and many had died in consequence of the insufficiency of Vitamin B and all must have had their vitality and therefore power of resistance to infection by parasites impaired. On the other hand, all the mice on the present occasion had eaten green stuff from an early age and all were extremely vigorous and full grown. It was noticeable in the mice of the previous occasion that they put on weight very quickly after being fed on lettuce and it is also noteworthy that the five large mice referred to above as only showing one case of infection with "*H. fraterna*" eggs, and the five young rats, had both received green food for at least three weeks previously to the egg-feeding.

ON THE IDENTITY OF *HYMENOLEPIS NANA* OF MAN WITH "*HYMENOLEPIS FRATERNA*" OF RODENTS.

My confirmation of the results obtained by Saeki[1] and the impossibility of distinguishing *Hymenolepis nana* from "*Hymenolepis fraterna*" of the rat and mouse, either in the adult form or in the egg, make it almost certain that these two parasites are one and the same, though found in two such dissimilar hosts. This conclusion is to a large extent confirmed by the geographical

[1] Professor Fülleborn has informed me verbally that he also has succeeded recently in infecting rats with human *H. nana* eggs.

distribution of *H. nana* and "*H. fraterna.*" These two parasites exist side by side in all tropical or sub-tropical countries (including the more southern of the United States which lie wholly in the same temperate zone as southern Europe), but *H. nana*, according to the statistics, is very rare in all the colder countries (*e.g.* northern half of Europe, Canada), except in a few localities, *e.g.* in the Liège district of Belgium, where, as Joyeux states, 0·1 per cent. of the miners are infected, the parasite persisting, with Ankylostoma, in the warmth and humidity of the mines. "*H. fraterna*," on the other hand, appears to be as common in temperate as in hot climates. This rarity of *H. nana* in Man in the colder climates however cannot, in the present state of knowledge, be adduced as an argument against the identity of *H. nana* with "*H. fraterna*," both for the simple reason that, owing to the total or almost total absence of dysentery, enteric, helminthiasis and other gut diseases in cold countries, routine faecal examinations are not so common as in warm regions, and therefore, in the frequent absence of acute symptoms, infection with *H. nana* is not so likely to be detected[1], and for the further reason that in many of these cold countries the conditions under which people live are more hygienic and rats and mice are not so abundant.

Apart from the single experiment of Grassi, who fed four adults and two children on "*H. fraterna*" eggs, one child only developing some fifty worms after fifteen days or so—an experiment not decisive owing to the non-exclusion of the possibility of the child having been naturally infected with *H. nana*—there is no direct evidence for the infestation of man or monkeys with "*H. fraterna*" eggs. Indeed, judging from the few experiments of Joyeux, Scott and myself (*vide infra*) it is extremely difficult to infect monkeys even with *H. nana* eggs, though Saeki claims to have succeeded in one case. Until therefore there exists good direct evidence that Man can become infected with *Hymenolepis* eggs from the rat or mouse, the conclusion that *H. nana* and "*H. fraterna*" are identical is not absolutely certain. The fact recorded by Grassi, however, that it is extremely difficult to infect wild rats with "*H. fraterna*" eggs derived from tame rats is a clear indication that the apparent unsusceptibility of monkeys (and Man?) to these same eggs is but of little importance, and the further fact that the eggs of such a distinct species as *Hymenolepis diminuta* can develop in Man under certain conditions renders it extremely probable that those of "*H. fraterna*" can—also under certain conditions. We conclude then provisionally that these dwarf Cestodes of Man and Rodents both belong to the same species, to both of which the name *Hymenolepis nana* alone is applicable, the name "*fraterna*" lapsing.

[1] But for its association with Ankylostomiasis, it is doubtful if the presence of *H. nana* in Liège would have been discovered.

ON THE DIMENSIONS AND MODES OF OCCURRENCE OF *HYMENOLEPIS NANA* AS FOUND IN TAME MICE GENERALLY.

On returning to England I handed the few specimens of *H. nana*, which I had obtained from the seven infected mice, to Dr H. A. Baylis, and he has kindly examined these for me and reported upon them in the paper preceding this (p. 415). It appears from this paper that at least three of the worms are larger than the remainder and the question arose as to whether the former are *H. nana*, or whether they belong to a distinct species: the "*H. longior*" which Baylis described in 1922. If the former were the case then the human *H. nana* must be capable of exceeding the 25 mm. length commonly assigned to it as a maximum (*vide* authorities quoted by Joyeux, 1920, p. 121); if the latter, then there must exist two species of dwarf tapeworm in Man as in Rodents and the maximum lengths (33–40 mm.) attributed to *H. nana* by Grassi and Calandruccio must refer to the larger species.

Knowing for a certainty that all the worms developed in my seven mice were derived from the eggs contained in the human faeces I employed and having some reason to doubt the existence of two distinct species of dwarf tapeworm (*H. nana* and "*H. longior*") in Rodents (and therefore in Man), I carefully examined the *Hymenolepis* worms contained in the hind intestines of 17 tame mice (66 examined). In the case of each mouse the eggs in the faeces were first examined and five or six of them measured, after which the last 15 cm. of the small intestine were carefully slit open, pinned out on wax (blackened) and then flooded with 0·5 per cent. saline. All the worms were thus floated and were clearly visible. The worms were detached, in most cases intact, and immediately fixed, extended on a slide, in hot 6 per cent. formalin and their lengths[1] measured. They were subsequently washed in water, graded up to 70 per cent. alcohol and left for a day or so, then graded down again to distilled water, stained overnight in very dilute Mayer's Acid Haemalum (transparent thin port-wine colour), washed for at least one hour in tap water, then graded up to Absolute, into xylol and mounted in balsam, great care being taken at all stages not to subject the worms to unnecessary motion. In this way I have obtained most sizes of intact mature egg-shedding worms, measuring (when mounted in balsam) from 7 mm. up to about 80 mm. in length, and *every size sheds the same type of egg* (outer envelope: max. diam. = 36·6–53·07; min. diam. = 25·6–43·92. Inner shell: max. diam. = 23·79–35·50; min. diam. = 20·13–31·11[2]), which is identical with the human *H. nana* type (outer envelope: max. diam. = 40·26–57·39; min. diam. = 34·77–54·90. Inner shell: max. diam. = 27·45–35·13; min. diam. = 23·79–33·67[3]).

[1] The worms contract greatly (× cir. $\frac{2}{3}$) when passing through alcohols and xylol. Thus my largest worm measured 120 mm. after fixation with hot formalin, but only about 80 mm. when mounted in balsam, and another worm which measured 28 mm. after fixation only measured 19 mm. in balsam. Compare the similar figures given by Joyeux for worms in physiological saline and alcohol respectively (54 > 35 and 61 > 39).

[2] Measurements of fresh eggs in faeces of tame mice.

[3] Measurements of formalin-preserved eggs from a child in Lisbon.

The lengths of some of these worms (from the scolex to the last gravid, usually the terminal, segment), intermediate between the minimum and maximum lengths just given, and the approximate number of segments[1] appertaining to some of the smaller specimens are as follows:

Approx. length of worm in balsam, in mm.	Maximum breadth in mm.	Approx. number of distinguishable segments	Approx. length of worm in balsam, in mm.	Maximum breadth in mm.
7	0·199	96	26	0·481
9	0·215	142	28	0·431
11	0·215	193	28	0·464
13·5	0·31	288	28	0·498
16	0·232	338	30	0·332
17·5	0·282	356	31	0·332
18	0·398	367	31	0·365
19	0·29	421	31	0·415
20	0·381	481	32	0·464
20	0·365	538	34	0·481
21·5	0·46	558	34·5	0·481
22	0·415	370	34·5	0·531
23	0·249	554	34·5	0·614
23	0·415	469	36	0·332
24	0·398	555	37·5	0·464
24	0·398	458	40	0·597
24	0·415	Not counted	41	0·498
24·5	0·415	—	45	0·514
25	0·481	—	51	0·415
26	0·464	—	54·5	0·614
			80	0·697

I therefore conclude, with Dr Baylis, that there exists but one species of dwarf tape-worm in the mouse—*H. nana*—and that individual sexually-mature worms may vary in length between 7 mm. and 80 mm. (lengths measured in balsam), a conclusion which agrees with that of Joyeux, who also found mature specimens of "*H. fraterna*" ranging from 7·37 mm. up to as much as 90 mm. in total length (when fixed in alcohol).

I may also mention that I subsequently examined another and still larger series of *H. nana* obtained from another lot of mice and obtained the same results. Both of these series of preparations were handed to Dr Baylis for examination and his conclusions are embodied in his paper on p. 415. "*H. longior*" therefore, like "*H. fraterna*," lapses as a distinct species.

It seems probable that the lengths of mature *H. nana* in the mouse (and in Man) are correlated with the degree of parasitisation of the host, as the following data from the 17 mice first examined by me suggest (all the lengths given are of specimens measured after fixation in hot 6 per cent. formalin, except those of A9, in which hot Schaudinn's fluid was used):

[1] These numbers of segments, which some authors lay stress upon, are, in my opinion, of little or no significance in view of the fact that at the base of the neck where the segments are extremely short and therefore numerous per millimetre length, they may be obvious in one worm and not in another; also in two worms of the same length and otherwise identical, proglottid formation may be slightly more advanced in one than in the other (as Joyeux remarks, the neck varies greatly in different individuals)—a trifling difference which however may add 50–100 segments to the credit of the former individual.

Label of mouse	Number of worms.	Lengths of worms in mm.
A30	1	*ca.* 120.
A51	1	*ca.* 118.
A62	1	*ca.* 78.
A9	2	*ca.* 60 and 48.
A40	6	*ca.* 76, 60, 53, 48 and 40 mm. (the sixth not measured).
A54	6	*ca.* 71, 67 and the other four (not measured) also large.
A56	6	*ca.* 85, 60, 54, 46 and the other two of similar lengths.
A42	7	*ca.* 71, 34, 29, 27 and one immature 14 mm. (two others not measured).
A60	7	*ca.* 80, 68, 65, 53, 50 and 43 mm. (one not measured).
A64	8	All large but only three measured: *ca.* 76, 59 and 48 mm.
A58	8 or 9	*ca.* 70, 63, 42 (others of moderate length not measured).
A22	About 20 worms.	Thirteen measured, which varied between 40 and 18 mm., all intermediate lengths being found.
A61	About 23 worms.	Twenty-one measured, comprising all lengths from 26 up to 53 mm.
A48	About 20 worms.	Twelve measured, comprising all lengths from 18 up to 58 mm.
A17	Innumerable.	Worms measured from 15 to 19 mm., with one exception which measured 27 mm.
A25	Innumerable.	All worms ranged between 10 and 30 mm.
A15	Innumerable.	All worms ranged between 9·5 and 16 mm.

From these data it would appear that in the case of *Hymenolepis nana*[1] the *length of the mature worm and the period of development vary inversely as the number of worms present in the host*[2]—a law which is in accordance with Herbert Spencer's more general law that relative lack of nutrition induces early sexual maturity and retards "vegetative" or body growth.

AN ATTEMPT TO INFECT TWO *CERCOPITHECUS* MONKEYS WITH HUMAN *H. NANA* EGGS.

On April 7th the Instituto Camara Pestana kindly purchased for me one young male *Cercopithecus* monkey. On April 8th and 9th I examined its faeces and only found some Nematode eggs. On the latter date, the monkey being firmly held and its jaws kept open, I squirted down its throat 6 or 7 c.c. of a strong centrifuged concentration of fresh *H. nana* eggs (derived from the faeces of a Lisbon child), which must have contained several hundreds. I also gave him a fig, the inside of which had been freely sprinkled with eggs, and this was eaten by the next morning. I also squirted on to his mouth several more c.c. of centrifuged egg concentration, a large part of which he licked off, and I repeated this last process on April 14th. This monkey was killed on April 24th, fifteen days after the first egg-feeding, when no trace of *Hymenolepis* worms could be found in the last 54 cm. of the small intestine. Another *Cercopithecus* monkey (a female, said to have been ten months old) was

[1] Professor Fülleborn informs me that this is also the case with *Clonorchis*.

[2] Only on one occasion did I find an exceptional condition. In this exceptional instance I found in one mouse one large worm (115 mm. long after hot formalin fixation) together with hundreds of minute immature worms—so numerous that they resembled long cilia lining the intestine. This was either a case of a second infection or of auto-infection, the eggs of the large worm developing in the same host, but, on this latter supposition, it is difficult to understand how the cysticerci of the young worms could have developed.

It is not improbable that in cases in Man in which only a few large *H. nana* were present, these were mistaken for small *H. diminuta* and recorded as such.

purchased on April 15th and similarly fed with *H. nana* eggs. This also failed to show any *Hymenolepis* infection on April 24th, nine days afterwards.

On an attempt to infect tame mice directly with the eggs of *H. diminuta* parasitic in tame mice.

Though the probabilities were all in favour of Joyeux's statement that the one-host life-cycle does not apply to *Hymenolepis diminuta* of the rat and mouse, yet confirmation was desirable, and I accordingly selected 50 mostly full-grown mice which had failed to show *H. diminuta* or *H. nana* eggs in their faeces after one examination and fed 24 of these mice on fresh *H. diminuta* eggs contained in the richly-infected faeces of two mice. Each of the 24 mice also ate a cube of bread soaked in milk and freely sprinkled with numerous *H. diminuta* eggs. The remaining 26 mice served as controls. I killed 20 of these egg-fed mice (4 had died) thirty-two days afterwards and failed to find a single case of infection with *H. diminuta*, though 8 contained *H. nana* worms and 6 had *Cysticercus fasciolaris* cysts in their livers.

In conclusion I wish to acknowledge my great indebtedness to Dr A. Bettencourt, Director of the Instituto Bacteriologico Camara Pestana at Lisbon, not only for all the facilities which made my work a success but also for his generosity in enabling me to visit southern Portugal (the Algarve province) in order to inspect the Bilharziasis centre at Tavira and in demonstrating to me personally his recent important work on this subject. I also wish to express my sincere thanks to Dr Luis Figueira for his uniform kindness and valuable assistance (linguistic and otherwise) during my stay, and to Dr Pereira da Silva for similar aid.

Summary of results and conclusions.

1. Out of 57 mice (previously ascertained to have been free from "*H. fraterna*") 30 were fed on fresh eggs of *Hymenolepis nana*, the other 27 serving as controls. Out of the 30 egg-fed mice, only 7 were found to be infected (23·3 per cent.) when examined from thirteen to twenty-six days afterwards. None of the 27 control mice showed infection.

2. This low percentage of infection (when compared with the 90 per cent. infection obtained in my previous work when I fed 20 mice on "*H. fraterna*" eggs) is probably due to one or more of three factors: (*a*) change of host (Grassi records that he found it very difficult to infect wild rats with "*H. fraterna*" eggs obtained from tame rats and in this case the animals were of the same or closely allied species); (*b*) the full-grown adult condition of the mice; (*c*) the perfect health of the mice, due to having been supplied with vegetable food (Vitamin B) every day, this condition rendering the mice refractory to infection. In my previous work the mice were in poor condition owing to deficiency of Vitamin B.

3. The proof that rats and mice can be infected with *H. nana* eggs from Man, the identity of structure of the two parasites and their eggs and their uniform geographical distribution render it practically certain that *H. nana* and "*H. fraterna*" are one and the same species. The fact that up to the present there is practically no evidence that Man can be infected with eggs derived from rats or mice does not militate against this conclusion.

4. Mature specimens of *H. nana* found in tame mice vary enormously in size (as already indicated by Joyeux)—between 7 mm and 80 mm. (measured in balsam) in length. "*H. longior*" (Baylis, 1922) does not exist as a distinct species.

5. I failed to infect two *Cercopithecus* monkeys fed with a plentiful supply of fresh *H. nana* eggs (from Man).

6. Experiments are described which confirm Joyeux's statement that *H. diminuta* (of the mouse), unlike *H. nana*, does not possess a direct one-host life-cycle.

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A NEW TREMATODE, *OCULOTREMA HIPPOPOTAMI* N.G., N.SP., FROM THE EYE OF THE HIPPOPOTAMUS.

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(With Plate XVI.)

The monogenetic trematodes have long been known as parasites of the lower aquatic vertebrates and many forms have been described from fishes, amphibians, and reptiles. They are largely ectoparasitic, although a few members of the group infest the oronasal cavities and urinary bladder of frogs and turtles. Up to the present there appears to be no record of their occurrence on either birds or mammals and consequently the unusual case here reported seems worthy of note. The material consists of five polystomes which bore the label, "from the eye of hippopotamus." Presumably they were collected by the late Professor A. Looss from a Nile hippopotamus in the Giza Zoological Gardens of Cairo, Egypt, but unfortunately no particulars are available. The specimens were given to me for identification by Dr Edward Hindle, recently Professor of Biology, School of Medicine, Cairo, to whom I wish here to express my thanks. Grateful acknowledgments are due also to Professor George H. F. Nuttall, Director of the Molteno Institute for Research in Parasitology, where the study was made.

The presence of parasitic worms in the organs of special sense is fortunately rare for in such delicate structures they may occasion the most serious effects. Especially is this true in the case of those species which habitually penetrate the tissues. The mammalian eye as a seat of parasitic infestation is a subject of much significance. The conjunctival sac affords an easy means of entrance and conditions there are favourable for the existence of parasitic forms. Diseases due to protozoans, insects, and insect larvae are not uncommon and medical literature records several instances of helminths in the eye. The most frequent affection is ocular filariasis, caused by *Loa loa*. Encysted *Paragonimus westermanii* and the larvae of various cestodes have been reported from the eyelids and orbit. The worms here described are ectoparasites that adhere to the conjunctiva and undoubtedly move about under the eyelids and on the anterior face of the eyeball. Since the holdfast organs of the polystomes are sufficiently powerful to maintain their attachment against the movements of

the lids they might frequently be the source of direct injury or cause lesions permitting the entrance and multiplication of pathogenic bacteria.

The specimens from the eye of the hippopotamus all contain eggs in the uterus and may be regarded as sexually mature, adult individuals. The largest measures 5·2 mm. in length and 2·2 mm. in greatest width; the smallest 3 mm. in length and 1·1 mm. in greatest width. All the worms are so contracted that the body wall is thrown into conspicuous folds or rugae which show that in the living condition they must have been capable of great extension. The body is flattened dorso-ventrally. It is oval to pyriform in shape, pointed anteriorly and attenuated posteriorly where it expands into the caudal disc or cotylophore which bears the posterior suckers. Behind the caudal limits of the vitellaria the body gradually narrows until it is almost circular in cross section anterior to the disc. In three of the worms the cotylophore is nearly terminal, in the other two it is inclined ventrally. Its width is less than that of the body and measures from 1 to 1·35 mm. There are six suckers arranged in a circle, each sucker adjacent to the one on either side of it. The central portion of the disc surrounded by the suckers is hardly as large as one of them. The suckers are set so that they open outward and caudad. They are constructed in three zones, arranged on a cuticular framework similar to that described (Stunkard, 1917) for the North American polystomes. The cuticular skeleton entirely separates the peripheral fibrous zone from the central fibrous zone (Fig. 4), and is regularly thickened into parallel rays that extend meridionally from the opening and maintain the globular form of the sucker. The suckers are almost spherical and measure 0·5 mm. in diameter while their openings vary from 0·05 to 0·125 mm. in diameter.

No hooks of any kind were observed. It is practically certain that great hooks are not present on the caudal disc of this species and since the larval hooklets of the polystomes are frequently shed, their absence is not surprising.

The cuticular covering of the body measures from 0·008 to 0·015 mm. in thickness. The muscles of the body wall are powerfully developed and both the circular and longitudinal fibres are visible in whole mounts. The circular or annular muscles are external, beneath them there is a sheet of crossed oblique fibres, and internally there are bundles of longitudinal fibres. The longitudinal fibres are especially developed in the posterior half of the body (Fig. 5). On the ventral side in this region branches from the longitudinal fibres form a median central column which passes into the cotylophore and there separates to be inserted into the bases of the suckers.

The oral sucker is slightly subterminal and like that of other polystomes is not sharply separated from the body parenchyma. It is almost spherical and so mobile that it may be longer in either the antero-posterior or lateral direction. It measures from 0·36 to 0·58 mm. in diameter. It is followed by the pharynx which is approximately the same size. There is a distinct oesophagus, which when the body is contracted forms a large cuticular lined sac

extending dorsally and ventrally. The caeca are simple unbranched tubes although their walls are much folded. In each of the specimens the caecum of the ovarian side terminates at the caudal margin of the vitellaria, whereas the caecum of the antovarian side extends farther caudad, terminating about one-third of the distance from the posterior margin of the vitellaria to the cotylophore. While the number of individuals is too small to be certain, it seems that this feature is one of considerable importance.

The excretory system is of the typical monogenetic form, arranged in duplicate, the structures on either side corresponding with those on the opposite side. The two excretory pores are situated on the dorsal surface about midway between the median plane of the body and the lateral margins, near the level of the caudal margin of the pharynx. They open from vesicular enlargements which, when empty, have folded walls. From each of these terminal vesicles a collecting duct, 0·03 to 0·06 mm. in diameter, extends ventrally and laterally, passing backward along the lateral aspect of the corresponding caecum (Fig. 6). Behind the vitellaria the ducts are much coiled (Fig. 5) and these coiled tubules extend to the posterior end of the body, where they wind about and then return anteriad. I have been unable to find any connections between the canals of the two sides although such communication probably exists. Due to the extreme coiling and intertwining of the descending and ascending ducts it is impossible without complete reconstruction to be sure into which the secondary tubules discharge.

The testis is situated about one-third of the body length from the anterior end. It is oval in shape, longer in the lateral axis of the body. It varies from 0·2 to 0·37 mm. in antero-posterior measurement, 0·7 to 0·9 mm. in width and in one specimen cut in cross sections it is 0·76 mm. in width and 0·66 mm. in thickness. The vas deferens arises at the anterior margin and loops dorsally over the median edge of the ovary (Fig. 3). Here it is a small tube 0·01 to 0·025 mm. in diameter. Anterior to the ovary it coils about, passing forward on the ovarian side of the body. As it approaches the level of the pharynx it turns abruptly ventrad and expands to a diameter of 0·06 to 0·09 mm. This portion and frequently also the entire duct is filled with spermatozoa. Near the ventral wall of the body this vesicular portion of the vas deferens communicates with a thicker-walled muscular portion of about the same diameter and 0·16 mm. long which forms a weak or rudimentary cirrus sac. The cirrus (Fig. 2) is a small papilla 0·05 mm. in length. No prostate cells were observed.

There is no genital sinus, the cirrus sac opening beside the uterus at the apex of a prominent genital eminence or papilla. There are minute cuticular thickenings in the form of longitudinal ridges on the lining of the cirrus sac, but no genital coronet or hooks.

The ovary is a curved cylindrical organ situated on the right side of the body immediately in front of the testis (Figs. 3, 6). It measures from 0·3 to 0·5 mm. in its dorso-ventral axis and from 0·14 to 0·2 mm. in diameter. The

ventral pole is filled with small cells which increase in size dorsally where fully formed ova are present. The oviduct arises from the median dorsal face (Fig. 6) and passes ventrad and posteriad. Here is gives off the genito-intestinal canal which passes by a winding course around the posterior side of the ovary and opens into the caecum of the ovarian side at about the level of the origin of the oviduct. Near the origin of the genito-intestinal canal the oviduct receives a short common vitelline duct after which it turns dorsally and passes forward. At or slightly anterior to the level of the ovary it turns toward the antovarian side of the body and opens into an expanded portion (Fig. 3) surrounded by the large cells of Mehlis' gland. This portion in each of the specimens contained an egg. From the glandular portion a narrow uterine duct passes ventrad toward the antovarian side where it opens into the uterus. This latter is a thin-walled tube which extends back to the testicular level and then forward to the genital pore; its terminal portion has strong muscular walls and opens beside the cirrus sac at the apex of the genital protuberance. In the different specimens the uterus contains from 4 to 12 eggs. These are oval, without caps or filaments, and measure from 0·23 to 0·27 mm. in length and from 0·12 to 0·14 mm. in width. The vitellaria consist of numerous small follicles which extend posteriad from the level of the pharynx one-half to three-fifths of the body length. Anteriorly they occupy the lateral fields and behind the testis the follicles of the two sides become confluent in the median line. Their position and extent is best shown in the figures. Anterior to the testis vitelline ducts from either side unite near the median line to form the common duct which joins the oviduct as previously described. There are no lateral vaginae.

The genus *Polystoma* at present contains a group of widely distributed species that manifest unusual morphological dissimilarity. This condition was discussed by the writer in an earlier publication (Stunkard, 1917). The polystomes are uncommon forms and although many species have been described only a few specimens have been found. Two species were described from single specimens and others from two or three. The small amount of material available for study has acted as a deterrent in the erection of new genera and it has been easier and simpler to include the previously described species in a single genus. The type of the genus is *Polystoma integerrimum*, parasitic in the urinary bladder of European frogs. In this species the uterus is long containing many eggs, the lateral vaginae produce prominent "Seitenwülste," the caeca are branched, the cotylophore bears great hooks and suckers without a cuticular skeleton. Two other species, *P. bulliense* Johnston 1912 and *P. alluaudi* Beauchamp 1913 have been reported from Amphibia, the former from Australia and the latter from Africa. In *P. bulliense* the caeca are unbranched and in *P. alluaudi* not only are the caeca simple but "Seitenwülste" and caudal hooks appear to be absent although the species was described from a single individual and consequently the description is incomplete. In the genus, so far as known, the long uterus with many eggs is

confined to species infesting the urinary bladder of amphibian hosts of the Old World. In respect to other characters, however, these amphibian forms of the Eastern hemisphere occasionally disagree with each other and agree with species parasitic in the urinary bladder and oronasal cavities of turtles in India, Europe, and North America. Consequently I am inclined to believe that the polystomes have a common ancestry and are genetically related. For the species from turtles, Ward (1917) proposed the subgenus Polystomoides with *P. coronatum* Leidy as type and undoubtedly this group is entitled to generic rank.

The species just described differs from all other known polystomes in many important features. Among them may be noted the reduction of cirrus sac and absence of a coronet of genital hooks, the absence of lateral vaginae, the short and unequal digestive caeca, the absence of vitellaria in the posterior portion of the body, and the extreme sinuosity of the excretory collecting ducts in this region. It would be impossible to include this species in either the genus *Polystoma* or *Polystomoides* and it must be regarded as type of a new genus in the family Polystomidae for which I propose the name *Oculotrema*. The characteristics mentioned may be accepted as the generic diagnosis. The genus is founded upon the species from the eye of the hippopotamus which is named *Oculotrema hippopotami*. The type specimen is deposited in the collection of the Molteno Institute for Research in Parasitology, Cambridge University, Cambridge, England.

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EXPLANATION OF PLATE XVI.

All figures are drawn from camera lucida tracings.

Fig. 1. *Oculotrema hippopotami*, from whole mount. Type specimen, ventral view. ×20.

Fig. 2. Frontal section through the genital protuberance showing cirrus and metraterm. ×130.

Fig. 3. Frontal section through the dorsal part of the body showing relation of organs. ×30.

Fig. 4. Frontal section through one of the caudal suckers. ×40.

Fig. 5. Cross section posterior to the vitellaria showing the musculature and the coiling of the excretory collecting ducts. ×40.

Fig. 6. Cross section at the level of the origin of the oviduct showing the relation of structures. ×40.

ABBREVIATIONS USED.

c.	excretory collecting tubule	*o.*	ovary
d.	vas deferens	*p.*	genital pore
e.	egg	*s.*	cirrus sac
g.	genito-intestinal canal	*t.*	testis
i.	intestine	*u.*	uterus
m.	metraterm	*v.*	vitellaria
Mg.	Mehlis' gland		

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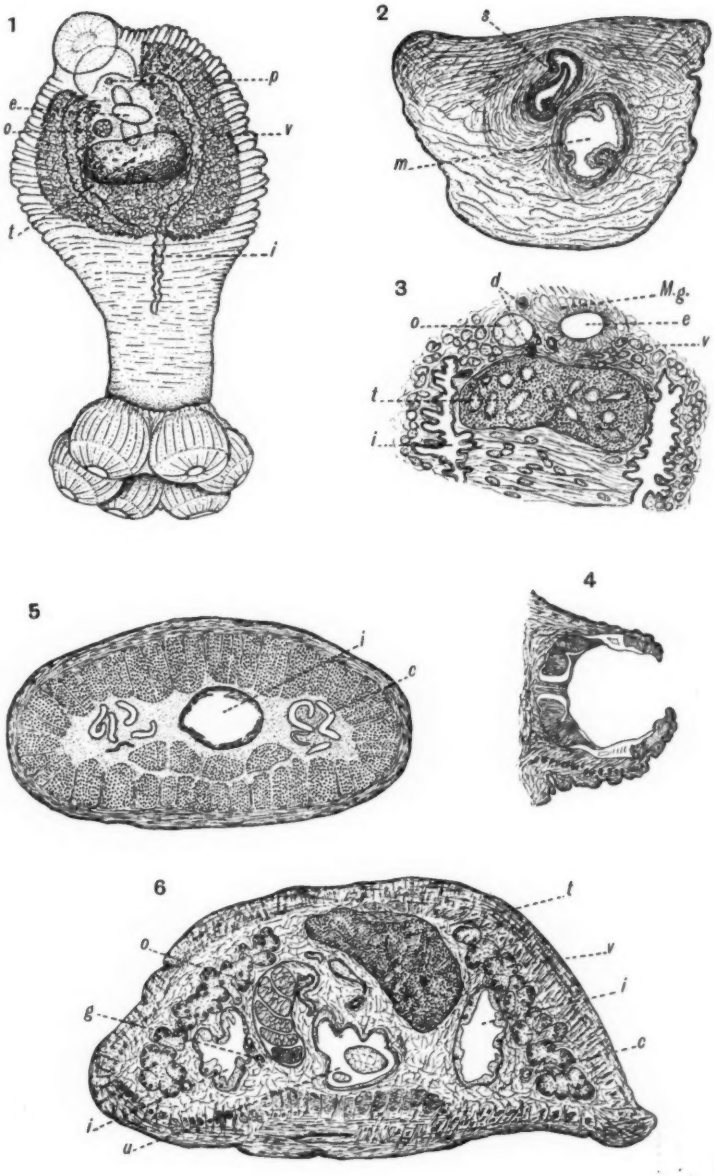
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ON A NEW BOTHRIOCEPHALUS AND A NEW GENUS OF PROTEOCEPHALIDAE FROM INDIAN FRESH-WATER FISHES.

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(With Plate XVII, including Figs. 1–35.)

Apart from the brief descriptions of Southwell (1913*a*, *b*; 1915*a*, *b*), little or nothing is known concerning the Cestode parasites of Indian freshwater fishes, and I have therefore the less hesitation in describing the following three new, and in some ways remarkable, species discovered in fishes common in the Ganges and Jumna at Allahabad, United Provinces, India.

Bothriocephalus (Rud. 1808; emend. Lühe 1899, 1910) *pycnomerus*, sp. n.

Of this new species I possess only two specimens, both of which were, while living, flattened between glass slides and fixed for about twelve hours in Mann's Fluid (distilled water 100 c.c., corrosive sublimate 2·5 gm., picric acid 1 gm., formol 10–25 c.c.) and preserved in 6 per cent. formalin. They were obtained from the extreme anterior end of the intestine of *Ophiocephalus marulius* Ham. Buch. (suborder Percesoces). One not-fully-mature specimen (which measured when alive about 45 mm. in total length—Fig. 1) was found in the intestine just behind the stomach, and the larger fully-mature specimen (about 76 mm. in total length when alive—Fig. 2) was found partly inside one of the two large pyloric caeca. I had preserved separately the unflattened scolex of the larger specimen but unfortunately it became lost: hence the description of the scolex is solely based upon the flattened scolex of the small specimen. These two worms belong to the genus *Bothriocephalus* (as restricted by Lühe 1910, and further amended in two particulars), and, though parasitic in a freshwater fish, they very closely resemble the *Bothriocephalus histiophorus* described by Shipley (1901) from the intestine of the marine sword-fish *Histiophorus* found in the Indian and Pacific Oceans.

The scolex of my smaller specimen is, when flattened, of the shape shown in Figs. 3 and 4. It is elongated (1·095 mm.[1] in length from apex to base) in form and measures 0·713 mm. in maximum breadth. The anterior third

[1] This and all subsequent measurements, it must be remembered, are made from flattened, *i.e.* distorted, specimens.

is narrower (0·489 mm.) than the dilated hind region and ends terminally in a truncated disc, the edges of which are drawn out into four lappets, as in Shipley's species, but, unlike Shipley's species, the four lappets are well armed with radially-disposed stout rod-shaped spicules (Figs. 4, 5). These spicules are, in my single specimen, 68 in number, *i.e.* about 17 to each lappet, and attain a maximum length of 73·2 microns in the middle region of the edge of each lappet but decrease in size down to 29·28 microns in the grooves between each pair of lappets. The broad hind region of the scolex shows two grooves representing shallow bothridia.

The strobila in these two flattened worms measures approximately 56 mm. (including the scolex) and 120 mm. (excluding the scolex and a few anterior proglottids) respectively. In the smaller specimen it attains a maximum breadth of 2 mm. and in the larger 3 mm. (parts of the strobila are much constricted). There is no neck region. The lines of demarcation of the proglottids, one from the other, vary greatly in distinctness and correspondence with the sets of genitalia in different regions of the strobila. Anterior to the first rudiments of the genitalia the flattened proglottids vary in width, in the younger worm, between 0·498 mm. and 1·693 mm. and in length between 0·099 mm. and 0·381 mm. In the region where the genital rudiments first become distinct, each apparent "proglottid" contains from two to four sets of genitalia and varies in length from 0·083 mm. to 0·448 mm. In the region of mature genitalia there is also often no exact correspondence between the outlines of the proglottids and the sets of genitalia. In certain places the "proglottid" appears to contain a single set of genitalia (Fig. 6) but in others the "proglottid" outline, when clearly distinguishable, may apparently contain two or even three genital sets (Fig. 8). In those regions of mature genitalia where there is apparently one genital set to each proglottid, the proglottids measure 1·826–1·992 mm. in breadth and 0·083–0·149 mm. in length. In the immature worm I counted in the last 36 mm. of the strobila 315 sets of genitalia. The proglottids therefore, counting one to each genital set, are extremely short, hence the name of the species: *B. pycnomerus*. In the region of gravid segments of the larger worm the major part of the series of proglottids is occupied by the large elongated uterine sacs (Fig. 13), and here again it is impossible to make the indistinct proglottids correspond with the uterine sacs. The proglottids in this region are 2·8–3·0 mm. broad, and the uterine sacs measure in length about 1·162 mm. and in breadth 0·198–0·448 mm. There is thus a considerable difference between the proglottid formation of this species and that of *B. histiophorus*, the mature and gravid proglottids of the former being more than twice as broad in proportion to their length as compared with those of the latter, and the segmentation much less distinct and definite.

The genitalia of *B. pycnomerus* are apparently constructed on much the same plan as those in Shipley's species. The two ovaries are narrow transversely-elongated bodies lying towards the hind end of the proglottid and

united in the median line by an "isthmus" (Fig. 10), from which arises the short oviduct which opens into the ootype, this last receiving the openings of the vagina and of the ducts of the shell-gland and vitellaria. From the ootype the tubular uterus arises in the usual way and after a convoluted course right and left, opens into the characteristic "uterushöhle" or uterine sac, a spacious transversely-elongated chamber which, as in *B. histiophorus*, opens either to the right or to the left of the median line on the ventral surface (Figs. 7, 9, 10). Neither the uterus sac nor its opening is ever situated in the median line (Figs. 7, 13), and apparently *B. pycnomerus* and *B. histiophorus* both differ from all other known species of *Bothriocephalus* in this character. As Shipley remarks, the uterine sac of each proglottid "is pushed sometimes to the right and sometimes to the left...and whether it lies to the right or to the left seems to follow no certain law." The vitellaria and vagina answer to Shipley's description for *B. histiophorus*, as also do the testes (apparently not more than 30–40 in each proglottid) and cirrus sac (which is large and muscular and contains several coils of the ductus ejaculatorius and in flattened transverse sections extends across about half of the depth of the proglottis—Fig. 11), though there is considerable variation as regards the relative antero-posterior positions of the openings of the cirrus and uterine sacs.

The eggs contained in the uterine sacs (Fig. 14) measure on the average 43·9 × 25·62 microns and are thus almost identical with the eggs of *B. histiophorus* (45 × 35 microns). The egg-shell is oval-shaped and thin, devoid of an operculum and with unstained granular contents containing nuclei. Hooks were absent.

A single excretory canal was situated on each side of the strobila.

It will thus be evident that *B. pycnomerus* chiefly differs from *B. histiophorus* in its armed scolex—a rare feature in species of *Bothriocephalus*—and its crowded segments.

Lühe's definition of the genus *Bothriocephalus* must, if it is to include the present new species, be amended to comprise armed as well as unarmed forms and forms in which the ventral uterine openings are a-median and irregularly alternate.

Southwell (1913*a*) has described from *Ophiocephalus striatus* and *Labeo rohita* (from Bengal) two specimens of what he identifies as "*Bothriocephalus* (*Ancistrocephalus*) *polyptera* Leyd.," i.e. *Polyonchobothrium polypteri*, the anatomy of which was described fully by Klaptocz in 1906. From the very few details supplied by Southwell we can safely say that his Bengal species is certainly not that originally described by Leydig, but that is all that can be said.

Gangesia wallago, gen. et sp. nov. Woodland 1924.

This species and the species next to be described are typical Proteocephalidae save that they possess conspicuous armed rostella—a combination of characters which, so far as I am aware, has not previously been described.

This fact will necessitate a slight emendation in the definitions of the Order Tetraphyllidea and the Family Proteocephalidae—a procedure which seems to me decidedly preferable to the alternative of the creation of a new family. I shall discuss the subject after the descriptions of the two species.

I possess about a hundred specimens of *Gangesia wallago*, mature and immature, flattened and unflattened, and some cut into transverse and horizontal serial sections, all obtained from the intestine (anterior, middle and posterior regions) of the Indian Siluroid *Wallago attu* Bleek. The larger of these Cestodes differ greatly in length, thickness and form (Figs. 15, 16) but I believe these differences are only due to local or general contraction, though at the time of collecting them they appeared to be separable into distinct kinds. My largest specimens were all flattened when living and it is difficult therefore for me to state the maximum length and breadth attained when alive. My largest unflattened specimen is about 18 mm. long and 0·5 mm. broad, whereas my largest *flattened* specimen measures (in balsam) about 70 mm. long and 1·8 mm. broad (dimensions which should probably be halved).

In many of my specimens the scolex has been torn off (Fig. 16) and there is then rendered visible (as also in flattened specimens) a very short neck region.

The scolex (Figs. 18, 19) measures 0·166–0·232 mm. in length and 0·298–0·448 mm. in breadth (all measurements of unflattened specimens). The four suckers are very mobile organs, with the cavities facing outwards and anteriorly and with distinct free edges, and measuring 0·120–0·172 mm. in breadth (between outer sides of the muscular walls). The "mouth" of the suckers is approximately circular in outline (Fig. 24) and two-thirds of the margin on the upper side and the adjacent area of the inner surface are armed with numerous closely-set minute spinelets (7·3 microns maximum length). These spinelets are not found elsewhere, either on the scolex, neck or anterior proglottids. There is a distinct rostellum which is protrusible beyond the suckers and which measures 0·132–0·199 mm. in breadth. It consists of a muscular bulb (Fig. 20) connected with longitudinal muscles at the base of the scolex and when half protruded the apex is saucer-shaped (Fig. 19) and is surrounded with a distinct margin which bears a single circle of conspicuous closely-set hooks of one kind only, 29·28–43·92 microns in length, of the shape shown in Fig. 21, and 28–42 in number. When the rostellum is fully protruded the terminal depression becomes everted (Fig. 17).

In specimens contracted anteriorly a neck is apparently absent but when extended a very short unsegmented, though often transversely wrinkled, neck becomes visible (Figs. 19, 16), with a maximum breadth of 0·332 mm. and passing imperceptibly into the anterior proglottids. The anterior immature proglottids in unflattened specimens are relatively very broad in proportion to their length (Figs. 15, 18), but the posterior proglottids are, proportionately to their breadth, much longer, and in mature worms the terminal proglottids may be one and a half times longer than broad. Most mature proglottids are

about twice as broad as they are long. The breadth of the proglottids in different regions varies greatly according to their state of contraction, but roughly speaking, in mature worms, the anterior proglottids just behind the neck measure 0·332–0·597 mm., and the ripe proglottids at the hind end of the strobila measure 0·747–0·996 mm. The total number of proglottids in the strobila of mature worms is large, well over one hundred and sometimes about two hundred, but of these only a few (rarely over thirty and usually fewer) are mature. The sexual openings are irregularly alternate and situated anterior to the middle transverse line of the proglottid. I could detect no uterine openings, though they probably exist.

The genitalia are typically Proteocephalid, and the figure (Fig. 22) I have provided renders a complete detailed description unnecessary. I will merely state the more important diagnostic features. The ovary is as usual bilobed, the two lobes being connected medianly by an isthmus. The uterus consists, also as usual, in early development of a median dorsally-placed pillar extending to the anterior limit of the proglottis, but in ripe proglottids lateral diverticula are developed on both sides from the median pillar, the total number varying between about 20–28. The testes lie in one continuous field (several layers deep (Fig. 23), anterior to the ovary, between this and the lateral strands of vitellaria), measure 65·8–109·8 microns in length, with a maximum breadth of 28 microns, and are over 100 in number. The vitellaria measure 20–99 microns in length, with a maximum breadth of about 18 microns. Both testes and vitellaria are medullary in position (Fig. 23). The cirrus sac and vagina openings lie, as already stated, usually well anterior to the middle transverse line of the proglottid, and they vary as to which is anterior. More usually in any given worm the cirrus sac opening is anterior to the vaginal, the reverse condition being either relatively rare or at most occurring half as frequently, but in some worms the vagina is either as frequently anterior as the cirrus sac or more so, and in some cases the two open in the same vertical plane, the vagina opening being the more ventral. The cirrus sac, when uneverted and uncontracted extends about one-third of the distance across the proglottis, and measures about 230 microns in length. The ductus ejaculatorius only forms coils when the sac is contracted.

In fully-mature proglottids the uterine diverticula are filled with numerous eggs, the three envelopes of which are apparently not completely developed (Fig. 34), judging from specimens obtained by teasing up preserved mature proglottids macerated in Marcacci's Fluid. On the other hand I found numerous spherical eggs with the three envelopes well developed on examining the contents of the intestine of the *Wallago attu* parasitized by the worm (Fig. 35). These eggs measured about 91·5–98·8 microns in external diameter (external thin envelopes) and the hookless embryos contained within the third innermost envelopes (the second and third envelopes are often apposed and so not distinct) measured 18·30–21·96 microns.

The excretory and nervous systems are apparently of the usual type.

The diagnostic characters of the new genus and species will be stated below.

Gangesia macrones sp. n.

This species was obtained from the intestine of the Siluroid *Macrones seenghala* Sykes. It is not common since I only found seven or eight worms in four fish out of about one hundred examined. These few specimens were all flattened between glass slides when alive, except the anterior ends, and consequently I can only state the approximate lengths of these specimens when unflattened, viz. about 28–56 mm., with a maximum breadth of a little over 1 mm. (Fig. 25). Two scolices only were obtained, and one of these I destroyed in an attempt to isolate the hooks on the rostellum, but the remaining scolex (unflattened) shows all features clearly (Fig. 26). It is 109·8 microns long (from the base of the globular rostellum to the apex) and 193·9 microns broad (between external sides of the suckers). A very conspicuous spherical muscular rostellum is present, measuring 109·8 microns in breadth, and this bears on its upper half a complete ring of hooks, about 33 in number. These hooks are of two kinds, large and small, which alternate in the ring, the larger measuring about 11·0–14·6 microns in length (from the convex edge of the base to the tip of the spine; the base measures about 9·5 microns in length and the spine 5·2 microns) and the smaller being about half this size, and of the shapes shown in Fig. 27. The four suckers are small (65·8–69·5 microns broad) and very thick-walled, with circular openings which face laterally and slightly anteriorly, and, as in *G. wallago*, the upper two-thirds of the margins and adjacent internal surfaces bear numerous minute spinelets.

A neck is altogether absent and the first proglottid is of about the same breadth as the scolex (0·204 mm.) and 0·054 mm. long. At about a quarter of the length of the strobila behind the scolex the unflattened immature proglottids are approximately square (0·249 mm. long and 0·265 mm. broad), and such is the case posteriorly, though in some cases the largest proglottids are distinctly broader than long (3·0 mm. broad and 1·8 mm. long when flattened). The terminal segment is always distinct, with a rounded hind border. The total number of proglottids in the strobila is between 150 and 200 or more. The genital apertures are irregularly alternate and are situated anterior to the middle transverse line of the proglottis. I could detect no uterine openings, though they probably exist.

The genitalia are typically Proteocephalid (Figs. 28, 29, 30) and similar to those of *G. wallago*. The two ovaries are connected by a very thin isthmus and are flatter in form than those of *G. wallago*. The uterine diverticula are about 20–30 in number. The testes lie in one continuous field, anterior to the ovary and between the lateral vitelline strands, and are over 100 in number. As in *G. wallago* the cirrus sac aperture is usually anterior to the vaginal opening but sometimes the reverse is the case. The uncontracted cirrus sac, in flattened worms, extends over only from one-sixth to one-quarter of the breadth of the

proglottis and is therefore, relative to the proglottis breadth, shorter than in *G. wallago*. The ductus ejaculatorius only forms coils inside the cirrus sac when the latter is contracted (cf. Figs. 31 and 32 of two cirrus sacs from two successive proglottids). One other point to be mentioned is that the vagina sometimes joins the ootype from the right side and sometimes from the left, apparently quite independently of the right or left position of the external openings of the genital apertures.

I neglected to obtain free eggs from the gut contents, and the eggs *in utero* are, as in *G. wallago*, not fully formed.

The diagnostic characters of this species will be stated below.

The Systematic Position and Diagnostic Characters of the Genus *Gangesia*.

Southwell (1913*a*, *b*), in describing what were almost certainly examples of the *G. wallago*[1] I have described above, which he found in the intestines of specimens of *Ophiocephalus striatus*, *Labeo rohita* and *Wallago attu*, from Bengal, unhesitatingly refers his specimens (apparently solely on the ground that the scolex possessed an armed "protrusible rostral disc") to the genus *Ophryocotyle*, and proceeds to remark upon the "considerable interest" which attaches to the presence of this genus in Teleost fishes (!), all other species having previously been found in birds. Had Southwell examined the internal anatomy of his specimens he would almost certainly have found himself in the predicament in which I found myself, viz. of having to classify a worm obviously Proteocephalid in its internal structure but possessing a scolex with a rostellum typically Cyclophyllidean in character. Both the Order Tetraphyllidea and the Family Proteocephalidae are at present so defined (Lühe 1910, La Rue 1914) as to exclude worms possessing rostella, and the existence of the two species just described opens up the question as to what classificatory value can be attached to the characters of the scolex in Cestoda. In Caryophyllaeidae I have recently shown (Woodland 1923, 1924) that the characters of the "head" are only specific in value, and most modern Helminthologists decline to follow the suggestion to place *Taenia saginata* in a genus (*Taeniarhynchus*) distinct from that which contains *T. solium* on the ground that while the former is altogether devoid of a rostellum and hooks, the latter possesses these structures in a very pronounced form. It would seem then that the presence or absence of a rostellum and hooks are, at least in Cyclophyllidea, merely characters of specific value and the two Proteocephalid Cestodes described in this paper should, according to this precedent, only be regarded as new species of the genus *Proteocephalus* Weinland 1858. But if the presence or absence of a complex structure like the muscular rostellum is only a character of specific value, it is certain that the taxonomic value of

[1] I think Southwell's wholly insufficient description of his "*Ophryocotyle bengalensis*" justifies me in not adopting his specific name for my type species of *Gangesia*. Only an adequate statement of distinctive characters can justly claim priority.

such characters as spinelets covering the scolex and anterior proglottids (*Acanthotaenia* von Linstow 1903), the small size of the scolex (*Ophidotaenia* Beddard 1913), re-entrant lower margins of the suckers (*Crepidobothrium* Monticelli 1899), the development of a circular fold of tissue at the base of the scolex which partly hides the suckers (*Choanoscolex* La Rue 1911) and irregular folds and lappets of tissue about the margin of the anterior surface of the scolex, which may enclose the suckers as in a corolla (*Corallobothrium* Fritsch 1886), is not greater, and, if this be so, the Proteocephalid genera named in brackets must all lapse (since they are all based upon these characters and other characters of still less importance) and all the species of the family Proteocephalidae will be grouped in one genus *Proteocephalus*. There is good reason for adopting this course, since the creation of separate genera, based on trivial differences, is extremely objectionable, involving as it does the artificial separation of forms closely allied to each other, which, as species of one genus, are naturally grouped together by the use of a common name. The alternatives then, in the case of the two species I have described, are either to regard them as new species of *Proteocephalus*, or to create a new genus for them and to recognize the genera above-named in brackets (excepting Beddard's *Ophidotaenia*[1]). If I followed my personal inclination I should adopt the former alternative, but until Helminthologists are willing to concur in such wholesale deletion the latter is the only practical one, even though the new genus created is solely distinguishable from the genus *Proteocephalus* (as defined by La Rue 1914) by the presence of an armed rostellum and the presence of spinelets in the suckers.

The new genus *Gangesia* may thus be defined: With the characters of the family Proteocephalidae but emended to include forms with armed rostella. With a scolex possessing a globose muscular rostellum, armed or unarmed[2], and no fifth sucker. The suckers may or may not[2] bear spinelets. Testes in a single broad field between vitellaria. Eggs with three membranes. Habitat: in freshwater fish.

The new species *G. wallago* is distinguished as follows: Length of strobila usually not exceeding 40 mm., with a maximum breadth of about 1·5 mm. Proglottids numerous, well over 100 in number in mature forms, narrow antero-posteriorly in front but square or elongated posteriorly. Segmentation distinct. Scolex 0·166–0·232 mm. long and 0·298–0·448 mm. broad. Suckers with projecting edges, 0·120–0·172 mm. broad, and in part bearing numerous closely-set spinelets. The globular rostellum bears a single circle of hooklets, all of one kind, 29·28–43·92 microns long and 28 to 42 in number. A very short neck is present but is only visible in specimens with the scolex torn off or in flattened specimens, and gradually increases in diameter up to the first

[1] Beddard's genus *Ophidotaenia* (founded on the presence of ventral uterine openings, which however are of common occurrence in other genera, musculature peculiarities, and inconspicuous scolices—Beddard 1913), Shipley's *Palaia* and Sonsino's *Pancerina* are all based on insufficient definitions and must be regarded as synonyms of *Proteocephalus*.

[2] A provision for future possibilities.

traces of segmentation. Genitalia like those of *Proteocephalus*. Uterine diverticula 20–28 in number. Testes over 100 in number, 65·8–109·8 microns in length and maximum breadth of 28 microns. Genital openings lie a little in front of the middle transverse line of the proglottid, and the cirrus sac and vaginal openings vary as to which is anterior. The uncontracted cirrus sac extends over about one-third of the distance across the proglottis. Eggs provided with three membranes, the outermost being 91·5–98·8 microns in diameter and the spherical embryo measuring 18·30–21·96 microns. Habitat: intestine of *Wallago attu* Bleek (and probably *Ophiocephalus striatus* and *Labeo rohita*), rivers of India.

The new species *G. macrones* is distinguished as follows: Length of strobila does not exceed 60 mm. in length, with a maximum breadth of about 1·2 mm. Proglottids numerous, between 150 and 200 in number in mature worms, very narrow antero-posteriorly in front but square or elongated posteriorly. Segmentation distinct. Scolex measures about 109 microns long and 193 microns broad. Suckers small and thick-walled, about 67 microns broad and bearing numerous closely-set spinelets on their upper edges and adjacent internal surfaces. The globular rostellum (about 109 microns in diameter) bears a single circle of hooks, of two kinds, large (11·0–14·6 microns long) and small (about 6 microns long) alternating. Neck absent. Genitalia like those of *Proteocephalus*. Uterine diverticula 20–30 in number. Testes over 100 in number. The genital apertures lie in front of the middle transverse line of the proglottid and usually the cirrus sac opening is anterior to the vaginal but the reverse condition also occurs. The uncontracted cirrus sac in flattened specimens extends over only from one-sixth to one-quarter of the breadth of the proglottis. Habitat: intestine of *Macrones seenghala* Sykes, from rivers of India.

The definitions of the Order Tetraphyllidea (as stated *e.g.* by Lühe 1910) and of the Family Proteocephalidae (as stated by La Rue 1914) must also be amended to include forms possessing definite muscular rostella, armed or unarmed.

In conclusion I wish to express my indebtedness to Dr H. A. Baylis for some suggestions and to Miss I. M. Bellis for assistance in originating new names from the Greek.

SUMMARY.

1. A new species of *Bothriocephalus*—*B. pycnomerus*—is described from the intestine of *Ophiocephalus marulius* Ham. Buch., from the Ganges and Jumna at Allahabad, India.

2. *B. pycnomerus* closely resembles *B. histiophorus* (Shipley 1901; from the marine sword-fish *Histiophorus*) in structure but differs chiefly in the possession of an armed scolex and its crowded indistinct segmentation.

3. Lühe's definition of the genus *Bothriocephalus* must be amended to include forms with armed scolices and forms in which the ventral uterine apertures are a-median and irregularly alternate.

4. Two new species of Proteocephalidae are described which possess armed muscular rostella, and for these a new genus *Gangesia* is created and defined. The definitions of the Order Tetraphyllidea (Lühe 1910) and of the Family Proteocephalidae (La Rue 1914) must be amended to include forms possessing muscular rostella, armed or unarmed.

5. These two new species—*Gangesia wallago* and *G. macrones*—were found in the intestines of *Wallago attu* Bleek and *Macrones seenghala* Sykes (both Siluroids) respectively, from the Ganges and Jumna at Allahabad.

6. Southwell's "*Ophryocotyle bengalensis*," from *Ophiocephalus striatus*, *Labeo rohita* and *Wallago attu*, is probably identical with *Gangesia wallago*.

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EXPLANATION OF PLATE XVII.

[All the drawings, with the few exceptions noted, made under the camera lucida.]

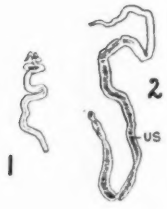
B, bothridial groove; *C*, cirrus; *CS*, cirrus sac; *EX*, common point of exit of the cirrus and vaginal apertures; *EXC*, excretory canal; *GP*, genital apertures; *NK*, neck (drawn out); *OV*, ovary; *R* rostellum; *RHK*, rostellar hooks; *SP*, scolex spicules; *TES*, testes; *UC*, uterine canal; *UO*, uterine aperture; *US*, uterine sac; *UT*, uterus; *VAG*, vagina; *VIT*, vitellaria.

Figs. 1–14. *Bothriocephalus pycnomerus* sp. n.

Figs. 1, 2. The two flattened specimens of *B. pycnomerus*, that of Fig. 2 being devoid of the scolex and some anterior proglottids. × ½ nat. size.

Fig. 3. Flattened anterior end, with scolex, of the small immature 45 mm. specimen. × 17·5 diam.

Fig. 4. Enlarged drawing of the flattened crown of the scolex, with its four armed lappets. × 56.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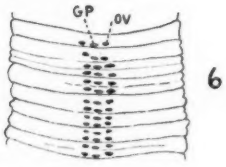
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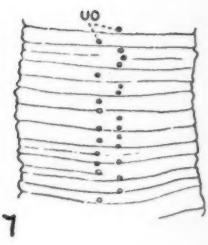
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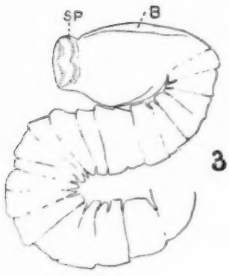
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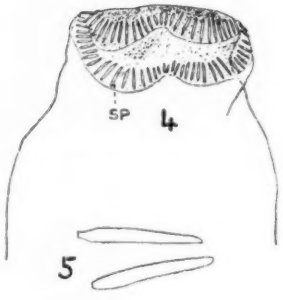
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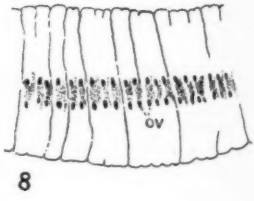
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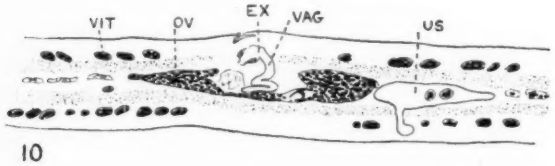
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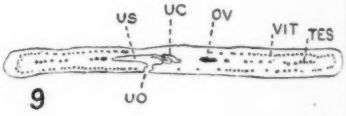
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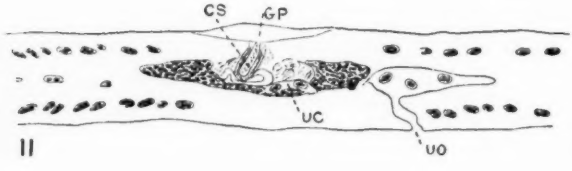
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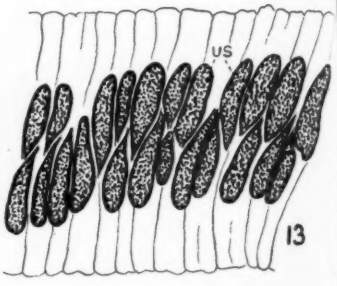
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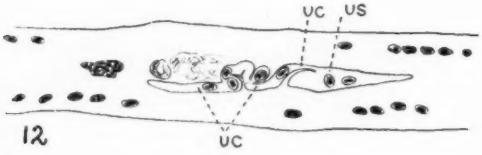
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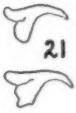
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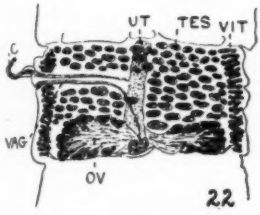

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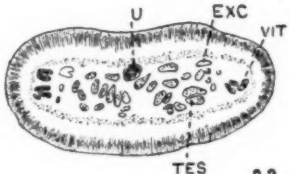

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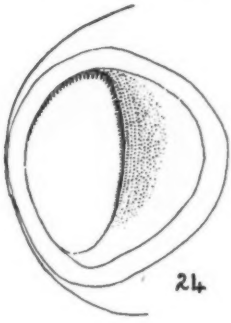
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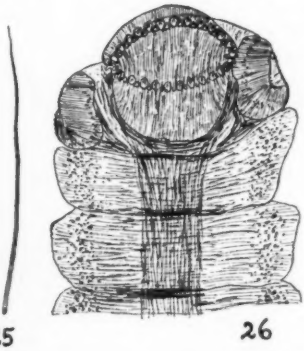
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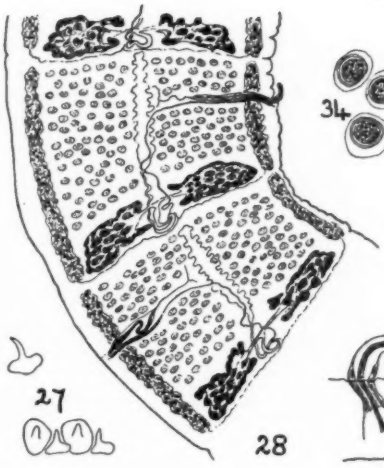
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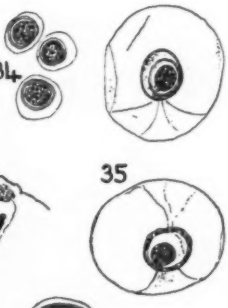
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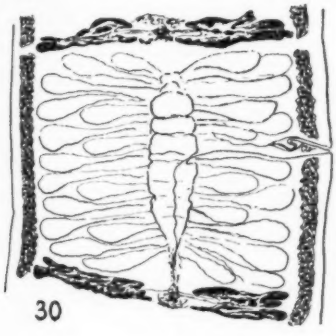
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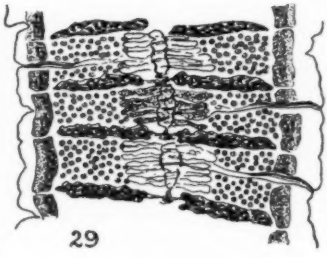
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